BONE MORPHOGENETIC PROTEIN-2 IN THE TREATMENT AND DIAGNOSIS OF CANCER

[0001] This application claims the benefit of U.S. Provisional Application No. 60/261,252 (Langenfeld), filed January 12, 2001.

FIELD OF USE

[0002] The present invention relates to the fields of molecular biology, immunology, and medicine and provides methods for the treatment and diagnosis of cancer. Specifically, it relates to the use of bone morphogenetic protein-2 (BMP-2), which is overexpressed in many common cancers as 1) a target for cancer treatment therapies and 2) a means to diagnose cancer.

BACKGROUND OF THE INVENTION

[0003] Various publications or patents are referred to in parentheses throughout this application. Each of these publications or patents is incorporated by reference herein. Complete citations of scientific publications are set forth in the text or at the end of the specification.

[0004] Lung cancer is the leading cause of cancer deaths in the United States with over 150,000 people this year expected to die from this disease (1). Despite improvements in diagnosis and treatment, only 10% of lung cancer patients survive 5 years (1) with the majority of patients succumbing due to spread of the tumor to other parts of the body. The genes that induce the invasion and metastasis of lung cancers are

poorly understood. Applicant's experiments to identify genes that regulate metastasis revealed that bone morphogenetic protein-2 (BMP-2) is overexpressed in human lung carcinomas. Subsequent experiments revealed that the protein is also overexpressed in many other common human cancers.

[0005] BMP-2 is a powerful morphogenetic protein that has been studied predominantly for its role in embryonic development and its ability to induce bone formation. The bone morphogenetic proteins (BMPs) are members of the transforming growth factor (TGF) superfamily, which are a phytogenetically conserved group of proteins (2). There are 20 isotypes of the BMPs with BMP-2 and BMP-4 sharing 92% homology (3). BMP-2 and BMP-4 are secreted proteins that induce pluripotential mesenchymal differentiation (4, 5) (6) and are required for the normal embryonic development of many organs including lung and bone (7, 8). BMP-2 can induce the entire developmental program of endochondral osteogenesis when introduced at an ectopic site (9). BMP-2 and BMP-4 also have chemotactic properties capable of inducing the migration of normal vascular endothelial and mononuclear cells (10, 11).

[0006] The BMPs are synthesized as inactive variable length precursor proteins (12, 13). The precursor BMP-2 proteins are proteolytically cleaved, producing a mature C-terminal 14-kDa protein that is the active peptide (9, 12). The mature BMP-2 protein signaling is mediated by transmembrane serine/threonine kinases called type IA, IB, and type II receptors (14-17). The receptor phosphorylates cytoplasmic targets, which includes the Smad family of proteins (18).

[0007] While BMP-2 expression has been noted in a few cancers, such as sarcomas (Guo, W., et al. "Expression of bone morphogenetic proteins and receptors in

sarcomas" Clin. Orthop. 365: 175-83 (1999))and pancreatic cancer (Kleef, J., "Bone Morphogenetic Protein-2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo" Gastroenterology 116: 1202-1216 (1999)) and in cancer cell lines (Hatakeyama, S., et al., "Expression of bone morphogenetic proteins of human neoplastic epithelial cells" Biochem Mol. Biol. Int. 42(3): 497 (1997)), inhibition of BMP-2 activity as a potential cancer treatment has neither been mentioned nor studied in the literature. To the contrary, several articles suggest that BMP-2 has an inhibitory effect on cancer cell proliferation and may be a useful therapeutic agent to treat cancer. (Kawamura, C., et al., "Bone morphogenetic protein-2 induces apoptosis in human myeloma cells with modulation of STAT3" Blood 96(6): 2005-11 (2000); Soda, H. "Antiproliferative effects of recombinant human bone morphogenetic protein-2 on human tumor colony-forming units" Anticancer Drugs 9(4): 327-31 (1998); Tada, A., et al., "Bone morphogenetic protein-2 suppresses the transformed phenotype and restores actin microfilaments of human lung carcinoma A549 cells" Oncol. Rep. 5(5): 137-40 (1998))

[0008] Applicant has discovered that expression of bone morphogenetic protein-2 (BMP-2) is linked to cancer invasion and growth and that inhibiting BMP-2 activity reduces the size of cancerous tumors in nude mice and down regulates the expression of VEGF and sonic hedgehog in lung cancer cell lines. Thus, the present invention is directed toward using BMP-2 as a target for cancer treatment therapies and as a means to diagnose cancer. Specifically, the therapeutic component of this invention involves administering to a patient a composition that inhibits bone morphogenetic protein-2 activity. The diagnostic component of the invention involves measuring the BMP-2 level in biological samples from both a patient and a non-cancerous subject and comparing

those levels, with elevated levels indicating cancer in the patient.

SUMMARY OF THE INVENTION

[0009] The present invention is related to Applicant's discovery that bone morphogenetic protein-2 (BMP-2) is overexpressed in many common human cancers and is linked to cancer invasion and growth. Further, inhibiting BMP-2 activity reduces the size of cancerous tumors in nude mice and down regulates the expression of VEGF and sonic hedgehog in lung cancer cell lines. Thus, the present invention pertains to the use of BMP-2 as a 1) a target for cancer treatment therapies and 2) a means to diagnose cancer.

[0010] A primary aspect of the present invention is to provide a method for the treatment of cancer by administering to a patient a therapeutically effective amount of a BMP-2 activity inhibitor. Some cancers that may be treated by this method are carcinomas, including, but not limited to, lung cancer, bladder cancer, breast cancer, colon cancer, kidney cancer, lung cancer, ovarian cancer, thyroid cancer, endometrial cancer, omental cancer, testicular cancer, and liver cancer. In a preferred embodiment of this invention the patient is human.

[0011] The BMP-2 inhibitor of this invention may be a polypeptide that binds specifically to bone morphogenetic protein-2, a polypeptide that binds specifically to a BMP-2 receptor, or an antibody that binds specifically to BMP-2. The BMP-2 inhibitor may also be an antisense oligonucleotide that binds to a BMP-2 nucleic acid sequence or some portion thereof.

[0012] This invention features several particular polypeptides that are BMP-2 inhibitors. Preferred embodiments of this invention feature known antagonists to BMP-2,

such as noggin, chordin, cerberus 1 homolog, gremlin, and DAN. Noggin is particularly preferred. Another aspect of this invention relates to the use of fragments of noggin, chordin, cerberus 1 homolog, gremlin, and DAN as BMP-2 inhibitors.

[0013] Another embodiment of this invention provides a method for treating cancer by administering to a patient a therapeutically effective amount of an expression vector encoding a BMP-2 inhibitor, such as a polypeptide that binds BMP-2 or an antisense oligonucleotide that binds to the nucleic acid for BMP-2. Another aspect of this invention includes the expression vector described above in which the nucleic acid sequence for BMP-2 is operably linked to a selective promoter. One preferred selective promoter encompassed by this invention is carcinoembryonic antigen promoter.

[0014] This invention also encompasses a kit that includes packaging material, a BMP-2 activity inhibitor, and instructions that indicate that the compound can be used for treating cancer in a patient. One type of cancer that may be treated is carcinoma. Particular carcinomas encompassed by this invention are lung cancer, bladder cancer, breast cancer, colon cancer, kidney cancer, ovarian cancer, thyroid cancer, endometrial cancer, omental cancer, testicular cancer, and liver cancer.

[0015] The diagnostic component of this invention includes a method for diagnosing cancer in a patient by obtaining a biological sample from a patient and measuring the level of BMP-2 in the biological sample, with an elevated level of BMP-2 indicating cancer in the patient.

[0016] Any assay available to measure BMP-2 levels is encompassed by this invention. Particularly preferred are immunoassays. Some examples of immunoassays included in this invention are Enzyme-Linked Immunosorbent Assay (ELISA), Western

blot, immunoprecipitation, in situ immunohistochemistry, and immunofluorescence. The Enzyme-Linked Immunosorbent Assay is most particularly preferred.

[0017] Another aspect of this invention is a method for the diagnosis of cancer in a patient by detecting overexpression of BMP-2 in the patient by (i) quantifying *in vivo* or *in vitro* the presence of BMP-2 in a patient or a biological sample obtained from a patient, (ii) comparing the result obtained in step (i) to that of a normal, non-cancerous patient, and (iii) diagnosing for the presence of cancer based on an increased level of BMP-2 in step (ii) relative to a normal, non-cancerous patient.

<u>BRIEF DESCRIPTION OF THE FIGURES</u>

[0018] Figure 1 illustrates representational difference analysis (RDA) subtraction. Figure 1(a) shows amplification of cDNA prior to subtraction. Lane 1: IHBE cells; lane 2: lung carcinoma. Figure 1(b) shows the distinct cDNA bands present after the second round of subtraction and amplification. Figure 1(c) lists the proteins that were identified by a BLAST data base search after the DNA corresponding to each of the bands shown in Figure 1(b) was isolated and sequenced.

[0019] Figure 2 is an ethidium-stained agarose gel showing the results of RT-PCR performed on human lung cancer specimens. Lanes 1-4 contain the results of the RT-PCR of various specimens, while lane 5 contains a marker.

[0020] Figure 3 illustrates Western blots showing mature BMP-2 overexpressed in lung cancer tissue specimens and lung cancer cell lines. Figure 3(a) is a representative Western blot showing overexpression of BMP-2 in cancer tissue specimens. Lanes 1-5: normal lung tissue, lane 6: SOAS osteosarcoma cell line, lanes 7-11: non-small lung cell carcinomas. Figure 3(b) is the corresponding actin immunoblot. Figure 3(c) is a Western

blot of non small cell lung carcinoma (NSCLC) subtypes. Lanes 1-4: normal lung tissue, lane 5: squamous carcinoma, lane 6: adenocarcinoma, lane 7: bronchoalveolar carcinoma, lane 8: large cell carcinoma. Figure 3(d) is the corresponding actin immunoblot. Figure 3(e) is a BMP-2 immunoblot of lane 1: benign lung tumor, lane 2: mesthotheleoma, lane 3: normal lung tissue, lane 4: carcinoid tumor, lane 5: normal lung, lane 6: NSCLC, lane 7: normal lung tissue, lane 8: NSCLC, lane 9: recombinant BMP-4. Figure 3(f) is a BMP-4-probed Western blot with the same lane contents as Figure 3(e), except lane 9, which is recombinant BMP-4. Figure 3(g) is the corresponding actin immunoblot.

[0021] Figure 4(a) is a Western immunoblot of total cellular protein that demonstrates that normal and malignant human lung cell lines express mature BMP-2 protein. Lanes (1) IHBE; (2) SOAS; (3) H7249; (4) A549. (b) Western blot of cell culture media shows lung cancer cell lines secrete a BMP-2 precursor protein. Lanes (1) lung cancer tumor specimen; (2) A549 media; (3) H7249 media; (4) IHBE; (5), NBE media; (6) serum free media alone. (c) immunoblot of BMP type IA receptor. Lanes (1-3) normal lung tissue specimens; (4) IHBE cells; (5) H7249 cells; (6) A549 cells; (7-9) lung cancer tissue specimens.(d) immunoblot of BMP type lB receptor. (1-3) normal lung tissue specimens; (4) IHBE cells; (5)H7249 cells; (6) A549 cells; (7-9) lung cancer tissue specimens.

[0022] Figure 5: 5(a) is an immunohistochemistry localizing BMP-2 expression to the tumor cells. BMP-2 expression in a NSCLC demonstrating cytoplasmic staining of the tumor cells (arrowheads). The nuclei (n) of the tumor cells and the interstitium (I) are non-reactive; (b) Preabsorption of the BMP-2 antibody with recombinant human BMP-2

is non-reactive with the tumor cells (arrows). Original magnification x 82 .

[0023] Figure 6(a) is a BMP-2 Western blot of human breast tumors and corresponding normal tissue. Lane 1: NSCLC, lane 2-5: breast carcinomas, lane 6-8: normal breast tissue, lane 9: recombinant BMP-2. Figure 6(b) is a BMP-2 Western blot of common human carcinomas and the corresponding normal tissue. Lane 1: normal endometrium, lane 2: endometrial carcinoma, lane 3: ovarian carcinoma, lane 4: normal colon, lane 5: colon carcinoma, lane 6: normal bladder, lane 7: bladder carcinoma.

[0024] Figure 7(a) is a Western blot showing BMP-2 expression in metastatic tumors. Lane 1: interstitial inflammatory lung disease, lane 2: normal omentum, lane 3: metastatic kidney tumor, lane 4: normal lymph node, lane 5: metastatic breast cancer, lane 6: metastatic kidney tumor, lane 7: metastatic NSCLC, lane 8: omentum carcinoma. Figure 7(b) is the corresponding actin immunoblot. Figure 7(c) is a BMPR IA Western blot, while Figure 7(d) is a BMPR IB Western blot. The contents of the lanes on both blots are the same: lane 1: normal kidney, lanes 2-3: normal lung, lane 4: metastatic kidney carcinoma, lane 5: metastatic breast carcinoma, lane 6: metastatic NSCLC, lanes 7-9: NSCLC. Figure 7(e) is BMPR IA Western blot and Figure 7(f) is a BMPR IB Western blot of common human carcinomas. Lane contents are the same on both blots: lane 1: normal kidney, lane 2: normal endometrium, lane 3: omentum, lane 4: normal colon, lane 5: ovarian carcinoma, lane 6: kidney carcinoma, lane 7: endometrial carcinoma, lane 8: omental tumor, lane 9: colon carcinoma.

[0025] Figure 8 is a Western blot showing BMP-2 in serum samples from lung cancer patients. Lanes 1-2: serum samples, lane 3: recombinant BMP-2.

[0026] Figure 9 shows that secreted BMP-2 precursor is proteolytically cleaved

by human leukocytes. Cell culture media from the A549 cells incubated with leukocytes for 16 hours is probed with BMP-2 antibody recognizing its mature C-terminal end. Figure 9(a) is the resulting Western blot: lane 1: A549 lysate, lane 2: media without leukocytes, lanes 3-4: media with human leukocytes. Figure 9(b) is the same immunoblot hybridized with BMP-2 antibody recognizing its N-terminal end. Figure 9(c) is a Western blot of leukocyte samples probed with anti-furin antibody.

[0027] Figure 10 shows that BMP-2 treatment enhances formation of blood vessels around a cancerous tumor. Each picture is of tissue from a nude mouse injected either with A549 cells or with A549 cells and BMP-2. The picture in the upper right shows tissue (including a tumor) from a nude mouse injected with A549 cells. Upper left: control. Upper right: mouse treated with BMP-2. Lower left: mouse treated with noggin.

[0028] Figure 11 shows tissue (from nude mice injected with A549 cells and nude mice co-injected with A549 cells and BMP-2) stained with anti-CD 31 antibody, which recognizes endothelial cells, viewed from under a microscope. Left: control. Right: BMP-2 treated.

[0029] Figure 12 shows that BMP-2 regulates sonic hedgehog expression. The Western blot on the left was probed with anti sonic hedgehog and shows an increase in sonic hedgehog expression as the amount of recombinant BMP-2 added to the A549 cell culture is increased. The Western blot on the right was probed with anti sonic hedgehog and shows A549 cell culture media without added noggin (Lane 1) and cell culture media with added noggin (Lane 2).

[0030] Figure 13 shows that BMP-2 stimulates the migration of A549 and H7249

human lung cancer cell lines. 13(a): Recombinant human BMP-2, 1 ng/ml, 10 ng/ml, 100 ng/ml, 500 ng/ml, or 1000 ng/ml was added to the lower well of the transwell chamber. Migrated cells counted using fluorescent microscopy. 13(b) Noggin inhibits BMP-2 induced migration. Lane (1), media alone; (2) recombinant BMP2 (500 ng/ml); (3) noggin (10 mg/mi) and recombinant BMP-2 (500 ng/ml). 13(c) H7249 cells migrated off cover slips towards Affi-Blue agarose beads containing recombinant BMP-2. 13(d) H7249 cells did not migrate off cover slips toward AffiBlue agarose beads containing dilution buffer. Similar results were found using the A549 cells. All the above experiments were repeated at least 3 times. Data presented as mean + standard deviation. 13(e): Recombinant human BMP-2 stimulates the invasion of A549 or H7249 cells. Recombinant BMP-2, 1 ng/ml, 10 ng/ml, 100 ng/ml, 500 ng/ml, or 1000 ng/ml was added to the lower wells of a Matrigel invasion chamber. Experiments were repeated at least 3 times. Data presented as mean + 5 standard deviation.

[0031] Figure 14(a) show tumor growth after 19 days following the subcutaneous co-injection of A549 lung cancer cells into nude mice with Affi-blue agarose beads coated with (1) 100 ug/ml of albumin, (2) recombinant human BMP-2, or (3) recombinant mouse noggin.

[0032] Figure 15 show that noggin inhibits VEGF expression in the A549 lung cancer cell line. The Western blot was probed with anti-VEGF antibody. The lane labeled with a plus was cell culture media from cultures treated with noggin. The lane labeled with a minus was cell culture media from control cultures.

DETAILED DESCRIPTION OF THE INVENTION

[0033] The present invention is related to Applicant's discovery that the

overexpression of bone morphogenetic protein-2 (BMP-2) is linked to cancer invasion and growth. BMP-2 is overexpressed in many common human cancers and regulates molecular pathways that are involved in the promotion of cancer. Inhibiting BMP-2 activity reduces the size of cancerous tumors in nude mice and down regulates the expression of VEGF and sonic hedgehog, which have been linked to cancer, in lung cancer cell lines. Thus, the present invention is directed toward using BMP-2 as a target for cancer treatment therapies and as a means to diagnose cancer.

[0034] The therapeutic component of this invention involves administering to a patient a composition that inhibits bone morphogenetic protein-2 activity. Such inhibition may be accomplished by ligands or antibodies that bind to BMP-2 or BMP-2 receptors. It may also be achieved by preventing the processing of pro-BMP-2, or blocking transcription or replication of BMP-2 DNA or translation of BMP-2 mRNA. Delivery of such compositions may be systemic or tissue-targeted.

[0035] The diagnostic component of the invention involves measuring the BMP-2 level in biological samples from both a patient and a non-cancerous subject and comparing those levels. Elevated levels of BMP-2 in the patient compared to the subject indicate cancer.

[0036] Although specific embodiments of the present invention will now be described, it should be understood that such embodiments are examples that are merely illustrative of a small number of the many possible specific embodiments that can represent applications of the principles of the present invention. Various modifications obvious to one skilled in the art to which the present invention pertains are within the spirit, scope and contemplation of the present invention as further defined in the

appended claims.

Definitions

[0037] A "bone morphogenetic protein-2 activity inhibitor" is a composition that antagonizes the activity of the BMP-2 protein by specifically binding to it or to BMP receptors, blocks the activation of pro-BMP-2, or prevents the replication or transcription of the BMP-2 gene or the translation of BMP-2 mRNA into protein.

[0038] "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. Polypeptide refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

[0039] An "expression vector" is a recombinant vector that incorporates the desired gene and associated control sequences that promote and/or regulate expression of the gene. The desired gene is "operably linked" to such control sequences. The term "operably linked" means that the regulatory sequences necessary for expression of the coding sequence are placed in an appropriate position in the expression vector relative to the coding sequence so as to enable expression of the coding sequence. The preparation of such recombinant expression vectors as well as the use of various control sequences is well known to those of skill in the art and described in many references. See, for example, Sambrook, J., et al., Molecular Cloning : A Laboratory Manual 2nd ed. (Cold

Spring Harbor, N.Y., Cold Spring Harbor Laboratory) (1989).

[0040] A "selective promoter" refers to a promoter that is not indiscriminately expressed. Instead it is expressed only, for example, in certain tissues, certain tumors, in response to certain treatments, or in response to certain events in a cell. Such tissue-specific, tumor-selective, treatment-responsive, or tumor endothelium directed promoters are described in Nettlebeck, D.M., et al., "Gene therapy: designer promoters for tumour targeting" Trends Genet 16(4); 174-81 (2000).

[0041] An "expression vector vehicle" refers to an expression vector paired with a moiety that facilitates delivery of the expression construct to cells *in vivo*. An expression vector may incorporate genes encoding the delivery moiety. One example of such an expression vector is a viral vector.

[0042] The term "antibody" refers to polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of a Fab or other immunoglobulin expression library.

[0043] "Polyclonal" refers to antibodies that are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen or antigenic functional derivative thereof. For the production of polyclonal antibodies, various host animals may be immunized by injection with the antigen. Various adjuvants may be used to increase the immunological response, especially when using an entire protein, or a larger section of the protein. The type of adjuvant used will depend on the hosts. Typical adjuvants include Fruend's, Fruend's complete, or oil-in-water emulsions. In these cases the entire protein or portion thereof can serve as the antigen. When a smaller peptide is utilized, it is advantageous to conjugate the peptide with a larger

molecule to make an immunostimulatory conjugate for use as the antigen. Commonly utilized conjugate proteins that are commercially available for such use include bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH).

[0044] "Monoclonal antibodies" are substantially homogeneous populations of antibodies to a particular antigen. They may be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture. Such methods are well known to those of ordinary skill in the art and include general hybridoma methods of Kohler and Milstein, Nature (1975) 256: 495-497, the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today 4:72 (1983) and the EBV-hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, pp.77-96, Alan R. Liss, Inc. (1985). The basic technique involves injecting a mouse, or other suitable animal, with an antigen. The animal is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells. The result is a hybrid cell, referred to as a hybridoma, that reproduces in vitro. The population of hybridomas are screened to isolate individual clones each of which secrete a single antibody species to the antigen. The individual antibody species obtained in this way are each the product of a single B cell from the immune animal generated in response to a specific antigenic site recognized on the antigen. Kohler, G. and Milstein, C. Nature (London) 256: 495-497 (1975) and Eur. J. Immunol. 6: 511-519 (1976).

[0045] The term "antibody fragment" refers to a portion of an antibody, often the hyper variable region and portions of the surrounding heavy and light chains, that displays specific binding affinity for a particular molecule. The term antibody fragment also includes single chain antibodies.

[0046] An "antisense oligonucleotide" is an oligonucleotide that specifically hybridizes, under cellular conditions, with the cellular mRNA or genomic DNA encoding a BMP-2 protein or some portion of such cellular or genomic DNA, thereby inhibiting biosynthesis of the BMP-2 protein. The binding may be via conventional base pair complementarity, or, in the case of binding to DNA duplexes, via specific interactions in the major groove of the double helix.

[0047] The term "effective amount" refers to the quantity of a compound that is sufficient to yield a desired therapeutic response without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention. The specific "effective amount" will, obviously, vary with such factors as the particular cancer being treated, the physical condition of the patient, the type of mammal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compounds or their derivatives.

[0048] A "patient" is a mammal suspected of having cancer. The patient is preferably human but may also be another mammal, such as a cat, dog, horse, cow, rat, or mouse.

[0049] A "biological sample" is a substance obtained from the patient's body. The particular "biological sample" selected will vary based on the cancer the patient is suspected of having and, accordingly, which biological sample is most likely to contain BMP-2.

[0050] An "elevated level" means the level of bone morphogenetic protein-2 that is greater than the level of analyte present in a particular biological sample of patient that

is not suffering from cancer.

[0051] A "carcinoma" is an epithelial cancer. Examples of carcinomas are bladder cancer, breast cancer, colon cancer, kidney cancer, lung cancer, ovarian cancer, thyroid cancer, endometrial cancer, omental cancer, testicular cancer, and liver cancer. The epithelium predominately lines ducts and lining of organs or glands.

BMP-2 as a Target in the Treatment of Cancer

[0052] The present invention is directed to the use of BMP-2 as a target in the treatment of cancer. Amino acids #283-396 of SEQ ID NO: 2 constitute the amino acid sequence of mature human BMP-2. Nucleotides #372-1514 of SEQ ID NO: 1 constitute the nucleotide coding sequence for human BMP-2. Given the experiments described above, any composition that 1) specifically binds BMP-2 or a BMP-2 receptor, thereby antagonizing BMP-2 activity, 2) blocks the processing of pro-BMP-2, or 3) prevents the replication or transcription of BMP-2 DNA or the translation of BMP-2 mRNA could be used as a therapy to treat cancer.

[0053] A compound that specifically binds to BMP-2 is any compound (such as a polypeptide or an antibody) that has a binding affinity for any naturally occurring isoform, splice variant, or polymorphism of BMP-2. As one of ordinary skill in the art will appreciate, such "specific" binding compounds may also bind to other closely related proteins that exhibit significant homology (such as greater than 90% identity, more preferably greater than 95% identity, and most preferably greater than 99% identity) with the amino acid sequence of BMP-2.

[0054] Similarly, a compound that specifically binds to a BMP receptor is any compound that has a binding affinity for any naturally occurring isoform, splice variant,

or polymorphism of the BMP receptor. As one of ordinary skill in the art will appreciate, such "specific" binding compounds may also bind to other closely related proteins that exhibit significant homology (such as greater than 90% identity, more preferably greater than 95% identity, and most preferably greater than 99% identity) with the amino acid sequence of a BMP receptor.

[0055] The present invention embodies polypeptides that specifically bind to BMP-2, thereby inhibiting its activity or that specifically bind to BMP receptors, thereby inhibiting BMP-2 activity. Specific embodiments of such polypeptides are described below.

[0056] The present invention encompasses known antagonists of BMP-2 activity, including noggin (Brunet, L.J., et al., "Noggin, Cartilage Morphogenesis, and Joint Formation in the Mammalian Skeleton" Science 280(5368): 1455-7 (1998); US Patent No. 6,075,007, Economides, et al.), chordin (US Patent No. 5,896,056, LaVallie , et al.; Millet, C., et al., "The human chordin gene encodes several differentially spliced variants with distinct BMP opposing activities" Mech. Dev. 106(1-2): 85-96 (2001)), gremlin (GenBank Accession No. AF154054), cerberus 1 homolog (GenBank Accession No. NM_005454), and DAN.

[0057] Recombinant mouse noggin from R & D Systems (Minneapolis, MN) was used in the inhibition experiments described in the Results section below. Mouse and human noggin share 98% homology. Therefore, this invention also relates to use of a polypeptide with the amino acid sequence of mature mouse noggin (amino acids #20-231 of SEQ ID NO: 6) and with the amino acid sequence of mature human noggin (amino acids #20-231 of SEQ ID No.: 4) as a BMP-2 activity inhibitor. The amino acid

sequence for human chordin is SEQ ID No: 8, for human gremlin is SEQ ID NO: 10, and for cerberus 1 homolog is SEQ ID NO: 12. The nucleotide coding sequence for human noggin is SEQ ID NO: 3, for mouse noggin is SEQ ID NO: 5, for human chordin is nucleotides #247-3114 of SEQ ID NO: 7, for human gremlin is nucleotides #130-684 of SEQ ID NO: 9, for human cerberus 1 homolg is SEQ ID NO: 11.

[0058] This invention also embodies polypeptide fragments of noggin, chordin, gremlin, cerberus1 homolog, and DAN that bind BMP-2 and inhibit its activity. Such polypeptides may be tested for inhibitory efficiency by culturing cells transformed with progressively shorter portions of the nucleotide sequences encoding the above proteins, recovering and purifying from the various cultures the resulting polypeptide, and testing those polypeptides for their ability to inhibit BMP-2 activity.

[0059] This invention also includes genetically altered BMP receptor proteins that inhibit BMP-2 activity. For example, altered BMP receptors that inhibit the binding effects of BMP-2 are described in U.S. Patent No. 6,291,206 (Wozney, et al.)

[0060] Also included by this invention are polypeptides that bind BMP receptors without activating them. (Nickel, J., et al. "The Crystal Structure of the BMP-2:BMPR-IA Complex and the Generation of BMP-2 Antagonists" The Journal of Bone & Joint Surgery 83-A, Supp.1, Part 1: 7-14 (2001). Kirsch, T., et al. "BMP-2 antagonists emerge from alterations in the low-affinity binding epitope for receptor BMPR-II" The EMBO Journal 19(13):3314-24 (2000)) Particularly preferred are ligands that will bind BMP IB receptors, as aberrant expression of the BMP IB receptor in many human cancer specimens has been noted, as discussed in the Results section below. (Ide, H., et al., "Cloning of human bone morphogenetic protein type IB receptor (BMPR-IB) and its

expression in prostate cancer in comparison with other BMPRs" Oncogene 13(11): 1377-82 (1997)). The coding sequence for BMP IB precursor is nucleotides #274-1782 of SEQ ID NO: 13. The amino acid sequence for BMP IB is amino acids #14-502 of SEQ ID NO 14.

[0061] This invention also encompasses expression vectors that incorporate a nucleotide sequence encoding an inhibitor of BMP-2 activity operably linked to control sequences that promote and/or regulate expression of the nucleotide sequence. The preparation of such expression vectors as well as the use of various control sequences is well known to those of skill in the art and is described in many references, such as Sambrook, et al. (1989). Expression vectors can be derived from bacterial plasmids, from bacteriophage, from transposon, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses and from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. Promoters can be prokaryotic, such as lacI, lacZ, T3, T7, gpt, lambda PR, PL, and trp, or eukaryotic, such as CMV immediate early, HSV thymidine kinase, early and late SV40, LTR's from retrovirus, and mouse metallothionein-1. Selective promoters such as those described in Nettlebeck, D.M., et al., "Gene therapy: designer promoters for tumour targeting" Trends Genet 16(4); 174-81 (2000) that are tissue-specific, tumor-selective, treatment-responsive, or tumor endothelium directed may also be used. For example, the promoter of the carcinoembryonic antigen (CEA) is expressed on many breast, lung, and colorectal cancers.

[0062] For introduction of a gene that encodes a protein that antagonizes BMP-2 activity an expression vector vehicle that will facilitate delivery of the desired gene to the

affected cells may be used. One way to facilitate delivery is by using an expression vector derived from virus. Examples of viral vectors that have been successfully used to deliver desired sequences to cells with high infection efficiency are adenoviral, retroviral, vaccinia viral, and adeno-associated viral vectors. Commonly used viral promoters for expression vectors are derived from polyoma, cytomegalovirus, Adenovirus, and Simian Virus 40 (SV40). It is also possible to use promoter or control sequences normally associated with the desired gene sequence, if such control sequences are compatible with the host cell systems.

[0063] Non-viral expression vector vehicles are also available. For instance, the expression vector could be associated with one or more lipids. As is known in the art of lipid-based gene delivery, such nucleic acid –lipid complexes can be in a variety of different forms depending generally on the nature of the lipid employed, the ratio of nucleic acid to lipid and /or other possible components, and the method by which the complex is formed. Examples of complexes include liposomes and micelles. Liposome-mediated gene transfer seems to have great potential for certain in vivo applications in animals. Studies have shown that intravenously injected liposomes are taken up essentially in the liver and the spleen, by the macrophages of the reticuloendothelial system. Using a catheter to introduce liposomes coupled to expression vectors to particular cellular sites has also been described. (Nabel, E.G., et al., Science 249:1285-1288 (1990))

[0064] Another possible expression vector vehicle consists of a cell receptor-specific ligand and a DNA-binding agent that would bind to the expression vector. (Nishikawa, M. et al., Gene Therapy 7:548-55 (2000)). Such a vehicle could also

comprise a cell receptor-specific ligand and the nucleic acid-lipid complex described above. (Nicolau, C. et al., Methods Enzymol 149: 157-76 (1987))

[0065] In addition, the present invention embodies antibodies that specifically bind BMP-2 or BMP receptors, thereby inhibiting BMP-2 activity. When raising antibodies to BMP-2 or BMP receptors, the entire protein (either the precursor or the processed protein), or a portion thereof, may be utilized. Information useful in designing an antigen for the production of antibodies to BMP-2 may be deduced by those of skill in the art by homology analysis of SEQ ID NO: 2, especially amino acids #283-396 of SEQ ID NO: 2.

[0066] A recombinant human BMP-2 protein is commercially available from R & D Systems (Minneapolis, MN) and portions of the BMP-2 protein may be produced by a variety of methods. In order to raise antibodies to particular epitopes, peptides derived from the full BMP-2 sequence may be used. Custom-synthesized peptides in the range of 10-20 amino acids are available from a multitude of vendors, and can be ordered conjugated to KLH or BSA. Alternatively, peptides in excess of 30 amino acids may be synthesized by solid-phase methods, or may be recombinantly produced in a recombinant protein production system. In order to ensure proper protein glycosylation and processing an animal cell system (e.g., Sf9 or other insect cells, CHO or other mammalian cells) is preferred.

[0067] Selection of antibodies which alter the activity of BMP-2 may be accomplished in several ways. Antibodies that alter the binding of BMP-2 to a receptor may be detected by well known binding inhibition assays. For instance, according to standard techniques, the binding of a labeled (e.g., flourescently or enzyme-labeled)

antibody to BMP-2, which has been immobilized in a microtiter well, is assayed for BMP-2 binding in both the presence and absence of the appropriate receptor. The decrease in binding will be indicative of a competitive inhibitor relationship between the antibody and the receptor. In addition, antibodies that are useful for altering the function of BMP-2 may be assayed in functional formats, such as the cell migration assays described in the Results and Examples sections.

[0068] This invention also embodies compositions that prevent the processing of inactive BMP-2 precursors. BMP precursors are proteolytically activated by proprotein convertases. For example, BMP-2 is cleaved by furin convertase from human leukocytes Furin inhibitors are known. See, e.g., Cameron, A., et al., "Polyarginines are potent furin inhibitors" J. Biol. Chem. 275: 36741-49 (2000).

[0069] While the BMP-2 inhibitors discussed above adversely affect BMP-2 activity after it is expressed, it will be readily apparent to one of ordinary skill in the art that specific prevention of BMP-2 biosynthesis will achieve the same goals as more direct inhibition of its activity. Consequently, this invention also encompasses inhibition of BMP-2 biosynthesis as a method for treating cancer. Such inhibition may be achieved by selectively degrading mRNA encoding BMP-2 or by interfering with transcription or translation of such mRNA. See Glavic, A., et al., "Xiro-1 controls mesoderm patterning by repressing BMP-4 expression in the Spemann organizer" Dev. Dyn. 222(3): 368-376. As mentioned above, BMP-2 shares 92% homology with BMP-4.

[0070] Inhibition of BMP-2 biosynthesis to treat for cancer could also be achieved through antisense therapy. Antisense therapy is the administration or in situ generation of oligonucleotides that specifically hybridizes, under cellular conditions, with

the cellular mRNA or genomic DNA encoding a BMP-2 protein or some portion of such cellular or genomic DNA, thereby inhibiting biosynthesis of the BMP-2 protein. Antisense therapy refers generally to the range of techniques known by one of ordinary skill in the art, and includes any therapy that relies on specific binding to oligonucleotide sequences.

[0071] Delivery of an antisense oligonucleotide of the present invention can occur in a variety of ways. For example, an antisense oligonucleotide can be delivered as an expression vector that produces RNA which is complementary to at least a unique portion of the cellular mRNA encoding BMP-2. Such an expression vector could be delivered to cells by one of the expression vector vehicles described above. Alternatively, the antisense oligonucleotide could be generated ex vivo as an oligonucleotide probe which, when introduced to the cell, inhibits biosynthesis of BMP-2 proteins by hybridizing with the mRNA or genomic sequences encoding BMP-2. Such oligonucleotide probes could be modified oligonucleotides that are resistant to endogenous nucleases and therefore are stable in vivo. General methods to construct oligomers useful in antisense therapy are known in the art. (Van der krol, et al., Biotechniques 6:958-976 (1988); Stein, et al., Cancer Res. 48:2659-2668 (1988).

[0072] Dosage forms of the BMP-2 inhibitors of this invention include pharmaceutically acceptable carriers known to those of ordinary skill in the art. Pharmaceutically acceptable components are those that are suitable for use with mammals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio. The carrier can be a solid or liquid and the type is generally chosen based on the type of administration being used.

The active agent can be coadministered in the form of a tablet or capsule, as an agglomerated powder or in a liquid form. Examples of suitable solid carriers include lactose, sucrose, gelatin and agar. Capsule or tablets can be easily formulated and can be made easy to swallow or chew; other solid forms include granules and bulk powders. Tablets may contain suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents and melting agents. Examples of suitable liquid dosage forms include solutions or suspensions in water, pharmaceutically acceptable fats and oils, alcohols or other organic solvents, including esters, emulsions, syrups or elixirs, suspensions, solutions and/ or suspension reconstituted from non-effervescent preparations reconstituted from effervescent granules. Such liquid dosage forms may contain, for example, suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, thickeners and melting agents. Parenteral and intravenous forms may also include isotonic salts and other materials to make them compatible with the type of injection or delivery system chosen.

[0073] For administration of an antibody to BMP-2, the pharmaceutically acceptable carrier will usually be an aqueous solution, such as normal saline or phosphate-buffered saline (PBS), Ringer's solution, lactate-Ringer's solution, or any isotonic physiologically acceptable solution for administration by the chosen means. In addition to additives for adjusting pH or tonicity, the antibody may be stabilized against aggregation and polymerization with amino acids and non-ionic detergents, polysorbate, and polyethylene glycol. Optionally, additional stabilizers may include various physiologically-acceptable coarbohydrates and salts. Also, polyvinylpyrrolidone may be added in addition to the amino acid. Suitable therapeutic immunoglobulin solutions,

which are stabilized for storage and administration to humans are described in U.S. Patent No. 5,945,098. Other agents, such as human serum albumin (HAS), may be added to the pharmaceutical composition to stabilize the antibody conjugates.

[0074] The method of administration can be any suitable method that effectively alleviates the particular cancer being treated. Possible methods of administration are oral, rectal, parenteral, enterical, subcutaneous, transdermal, peritoneal, intratumoral, or intravenous.

[0075] Any suitable dosage of the compounds may be given in the method of the invention. Dosage levels and requirements are well-recognized by those of ordinary skill in the art. As one of ordinary skill in the art will appreciate, an amount constituting an effective amount will vary depending on particular factors. For instance, specific dosage and treatment regimens will depend on facts such as the patient's general health profile, the type of cancer being treated, the severity and course of the patient's disorder, other therapeutics being administered to treat the cancer, and the judgment of the treating physician.

[0076] The present invention also provides kits for treating cancer using BMP-2 activity inhibitors. For example, such kits can comprise any one or more of the following materials: packaging material, at least one type of BMP-2 activity inhibitor, and instructions regarding dosage, method of administration, or the like for using the inhibitor to treat cancer.

Detection of BMP-2 to Aid in Diagnosis of Cancer

[0077] In addition to its therapeutic aspects, the present invention also relates to a diagnostic method for detecting the presence of elevated levels of BMP-2 in the patient.

Applicants have shown that BMP-2 is expressed in many common cancers. Elevated levels of BMP-2 can be detected in various biological samples in mammals, preferably humans. Applicants have shown the presence of BMP-2 in the blood serum of a human patient with cancer. Biological samples, including but not limited to blood, vitreous humor, sputum, aqueous humor, synovial fluid, urine, ascites, and tissue, will be drawn from the patient using standard techniques. Particularly preferred are serum samples.

[0078] The measurement of BMP-2 levels may be monitored using any method possible to detect BMP-2 in biological samples. Immunoassays, such as Enzyme Linked Immunological Assay (ELISA), Western blots, immunoprecipitation, in situ imunohistochemistry, and immunofluorescence assays are preferred. ELISA is particularly preferred. For a review of general immunoassays, see Stites, D.P., et al., eds., Basic and Clinical Immunology, 8th ed. (Appleton & Lange, Norwalk, Conn.) (1994). Immunological binding assays (or immunoassays) typically use an antibody that specifically binds to a protein of choice, BMP-2, in this case. The antibody is generally fixed to a substrate such as a plate or a column via covalent or non-covalent linkages (e.g., stretpavidin, protein A, protein G, secondary antibodies). Immunoassays also often use a labeling agent to specifically bind to and label the complex formed by the antibody and antigen. The labeling agent may be a labeled anti-BMP-2 antibody. Alternatively, the labeling agent may be a third moiety, such as a secondary antibody, that specifically binds to the antibody/antigen complex.

[0079] The immunoassays of this invention may be competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of antigen is directly measured. In a "sandwich" assay, for example, the anti-BMP-2

antibodies can be bound directly to a solid substrate on which they are immobilized. These immobilized antibodies then capture BMP-2 in the test sample. BMP-2 thus immobilized is then bound by a labeling agent, such as a second antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second or third antibody is typically modified with a detectable moiety, such as biotin, to which another molecule specifically binds, e.g., streptavidin, to provide a detectable moiety. Methods of binding molecules to a solid support, either covalently or non-covalently, are well known to those of skill in the art. A variety of solid supports known to those of skill in the art, e.g., plate, columns, dipsticks, membranes, and the like, can be used with the present invention.

[0080] In competitive assays, the amount of BMP-2 is measured indirectly by measuring the amount of a known modified BMP-2 displaced from a BMP-2 antibody by the unknown BMP-2 in a sample. In one competitive assay, a known amount of modified BMP-2 is added to a sample and the sample is then contacted with an anti-BMP-2 antibody. The amount of known modified BMP-2 bound to the antibody is inversely proportional to the concentration of BMP-2 in the sample. The amount of modified BMP-2 may be detected by providing a labeled modified BMP-2 molecule.

[0081] The label used in the assay is not a critical aspect of the invention, so long as it does not significantly interfere with the specific binding antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, or chemical means. Examples of such

labels are magnetic beads, fluorescent dyes, radiolabels, enzymes, and calorimetric labels such as colloidal gold or colored glass or plastic beads.

[0082] The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions. Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule, such as biotin, is covalently bound to the molecule. The ligand then binds to another molecule, such as streptavidin, which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a flourescent compound, or a chemiluminescent compound. The ligands and their targets can be used in any suitable combination with antibodies that recognize BMP-2. The molecules can also be conjugated directly to a signal generating compound, e.g., by conjugation with an enzyme or fluorophore.

[0083] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting flourescence. The flourescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers or the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels may be

detected simply by observing the color associated with the label.

[0084] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies.

RESULTS

[0085] Experimental results supporting the above uses of BMP-2 and its inhibitors are set forth in detail below. All of the experimental methods mentioned in this section, such as representational difference analysis, Western blot assays, and immunohistochemical studies, are described in detail in the Examples section that follows.

Identification of BMP-2 Using RDA Subtraction Technique:

[0086] Initially, Applicant performed representational difference analysis (RDA) on cDNA derived from normal and cancerous lung tissue samples to identify genes that were uniquely or highly expressed in human lung cancer in comparison to normal tissue. RDA has been described in the literature and allows detection of differences in gene expression between two similar populations. It involves exposing digested tester cDNA ligated to a primer to high concentrations of similarly digested but non-primer bearing driver cDNA, melting the tester and driver cDNA, and allowing them to hybridize. Subsequent PCR results in exponential amplification of the target cDNA of the tester that hybridizes to other tester cDNA. (Hubank, M., Nucleic Acids Research 22:5640-5648 (1994)) Here, Applicant used a non-small cell lung carcinoma (NSCLC) as the tester and immortalized human bronchial epithelial (IHBE) cells as the driver. IHBE cells rather than normal lung tissue were used, as IHBE cells proliferate at a rate that is more similar to human lung carcinomas than to normal lung tissue. Thus, Applicant avoided

identifying genes involved in the proliferation cascade but that were not by themselves transforming.

[0087] After two rounds of subtraction, several distinct bands, which were cloned and sequenced, were present in the amplified tester cDNA. (Figure 1b) A BLAST data base search identified BMP-2 expression in the lung tumor tissue specimen as well as expression of alpha-1-antitrypsin, cytokeratin 6, and lambda light. (Figure 1c)

Expression of BMP-2 In Various Cancer Tissue Specimens, Cancer Cell Lines, and Blood Serum from a Cancer Patient

[0088] Using reverse transcriptase polymerase chain reaction (RT-PCR), Western blots, and immunohistochemical assays to study the expression of BMP-2 and its receptors in various tissue specimens and in cell lines, Applicant found that BMP-2 was highly expressed in many types of cancers.

[0089] Applicant performed his initial experiments on normal and cancerous lung tissue and lung cancer cell lines. RT-PCR was performed using BMP-2 primers and showed expression in 9 out of 10 tumors examined. (Figure 2) Using Western blot analysis, Applicant found that the mature active 14 kD BMP-2 protein was aberrantly expressed in almost all of the 25 non-small cell lung carcinoma (NSCLC) tissue specimens examined There was little to no expression of BMP-2 in 11 normal lung tissue specimens. A representative Western blot is shown in Figure 3. An anti-actin immunoblot showed near equal loading of the samples. (Figure 3(b)) In addition, BMP-2 was found to be highly expressed in all epithelial derived lung carcinomas of which NSCLC is derived and in the rare malignant neuroendocrine tumor. (Figure 3(c) and Figure 3(e), Lane 4, respectively) Western blot analysis of each of the different cell types comprising NSCLC – adeno, squamous, large cell, and bronchoalveolar carcinomas –

revealed that the level of BMP-2 expression was not dependent on the cell type or whether the tumor was well or poorly differentiated. In comparison, the level of BMP-2 expression in benign lung tumors (Figure 3(e), Lane 1) and inflammatory diseases of the lung (Figure 7(a), Lane 1) was very low, similar to that seen in normal lung tissue, showing that BMP-2 is not an acute phase protein and that high levels of BMP-2 expression are indicative of malignant tumors. Neither BMP-4 nor BMP-7 expression was detected in the lung tissue specimens or the A549, H7249, IHBE, and NBE cell lines by Western analysis. (Figure 3(f))

[0090] Applicants also tested for expression of BMP-2 in various lung cancer and normal cell lines. Although the mature BMP-2 protein was detected in the cell lysate of the A549 and H7249 human lung cancer cell lines, the level of expression was not significantly different from the level of expression in the cell lysate of immortalized normal human bronchial epithelial cells (IHBE). (Figure 4(a)) Because BMP-2 is a secreted protein, Applicant also examined its expression in the cell culture media. A Western blot of the cell culture media showed the A549 and H7249 cell lines secreted a 43 kD BMP-2 precursor protein. (Figure 4(b), Lanes 2-3) This BMP-2 precursor was not detected in the media from either the IHBE or normal bronchial epithelial (NBE) cells (Figure 4(b), Lanes 4-5).

[0091] Immunohistochemistry studies of patient derived NSCLC also localized the expression of BMP-2 to the cancer cells (Figure 5(a)). Absorbing the anti-BMP-2 antibody with recombinant human BMP-2 completely inhibited staining of the tumors (Figure 5(b)). BMP-2 expression was not detected in normal lung tissue by immunohistochemistry.

[0092] Applicants turned next to receptors and found that normal and cancer lung tissue specimens and cell lines express both type IA and IB BMP receptors. The lung cancer and normal lung tissue specimens express a 55 kD and 44 kD type IA BMP-2 receptor. The tumor specimens expressed predominately the 55 kD receptor, while normal lung tissue specimens expressed a higher percentage of the 44 kD receptor. The A549, H7249, and IHBE cells only expressed a 44 kD type IA BMP receptor. (Figure 4(c))The tissue specimens and cell lines expressed a 44 kD type IB BMP receptor with normal lung tissue demonstrating more expression than that of the tumor specimens. (Figure 4(d))

[0093] Similar to their findings with lung tissue, Applicants found that BMP-2 was expressed in many other common human malignancies but not in their corresponding normal tissues. Western blot analysis revealed that BMP-2 was overexpressed in breast, bladder, colon, endometrial, omental, and kidney carcinomas with low levels of BMP-2 expression in the corresponding normal tissue. (Figures 6(a) and (b)). BMP-2 was also found to be expressed in ovarian (Figure 6(b), lane 3), mesothelioma (Figure 3(e), lane 2), thyroid, hepatoma, and testicular carcinoma.

[0094] BMP-2 and its receptors were also examined in both primary and metastatic carcinomas that were surgically removed from patients. BMP-2 was found to be highly expressed in kidney tumors that had metastasized to the lung, a metastatic breast cancer to chest wall cavity, and a NSCLC lung tumor that had metastasized to a regional lymph node. (Figure 7(a)) The BMP IA receptor was expressed equally between the primary and metastatic carcinomas and the corresponding normal tissue (Figure 7). The BMP IB receptor was expressed in all metastatic and primary tumors

examined. (Figure 7) The BMP IB receptor, in contrast to the BMP IA receptor, was not expressed in all the corresponding normal tissues. While it was expressed in normal lung tissue with slight expression in normal endometrium it was not expressed in normal kidney, colon, and omentum. (Figure 7(f)) Interestingly, the IB receptor was expressed in both primary and metastatic renal carcinoma, but not in normal kidney tissue. (Figure 7(f), Lane 6)

[0095] BMP-2 expression was also found in blood serum samples from lung cancer patients. (Figure 8)

Processing of Inactive BMP-2 Precursors

[0096] Because BMP precursors are proteolytically activated by proprotein convertases, Applicant studied whether BMP-2 could be processed following secretion, hypothesizing that secreted BMP-2 precursors from tumor cells may be processed by cells present in the tumor stroma. Because leukocytes normally infiltrate lung and furin convertase is ubiquitously expressed, the ability of leukocytes to cleave proprotein BMP-2 secreted from A549 cells was examined. First, Applicant determined that the furin convertase is expressed in human leukocytes isolated from whole blood. (Figure 9(c)). Human leukocytes were incubated with A549 cell culture media containing the BMP-2 precursor protein. A Western blot of the incubated media samples was probed with an anti-human BMP-2 precursor antibody that recognizes its C–terminal end. The 45 kD BMP-2 precursor protein was consistently decreased following incubation with the leukocytes (Figure 9(a)). By probing immunoblots with an anti-human BMP-2 antibody that recognizes its N-terminal end, Applicant identified a 31 kD BMP-2 product present only in the media samples incubated with leukocytes. (Figure 9(b)) This data shows that

BMP-2 precursor proteins are cleaved by human leukocytes.

Effect of BMP-2 on Tumors and Cancer Cell Lines

[0097] After determining that BMP-2 was highly expressed in most common cancers, Applicant performed experiments to show that BMP-2 causes cancer invasion and metastasis. Applicant performed experiments with lung cancer cell lines and with nude mice injected with A549 cells.

[0098] The experiments with the nude mice showed that BMP-2 treatment enhances blood vessel formation around tumors from nude mice injected with A549 cells. Some of the mice were co-injected with BMP-2. Gross observations of tissue harvested after six days showed that the addition of recombinant BMP-2 to developing tumors in nude mice caused increased blood vessel formation. (Figure 10) Tissue was also stained with anti-CD 31 antibody which recognizes endothelial cells. A person blind to how the tumors were created then observed the tissue through a microscope and counted the number of vessels that had formed in the tumor. This data showed that BMP-2 caused a statistically significant increase in the number of blood vessels in the tumor. (Figure 11)

[0099] Other studies showed that addition of BMP-2 to cancer cell lines increased expression of vascular endothelial growth factor (VEGF) and the oncogene Sonic Hedgehog. VEGF is the most potent angiogenic factor and is though to be essential for tumor growth and metastasis. (Folkman, J. J. Nat'l Cancer Inst. 82:4 (1990); Zetter, B. Annual Rev. Med. 49:407 (1998); Ferrara, N. Current Topics Microbiol. Immunol. 237:1 (1999)) Transgenic mice studies have confirmed that overexpression of sonic hedgehog can cause tissue-targeted cancer. (Oro, A.E., et al., "Basal carcinomas in mice overexpressing sonic hedgehog" Science 276: 817-21 (1997)) The addition of

recombinant BMP-2 to human aortic endothelial cells in culture caused an increase in VEGF secretion as determined by ELISA performed on the cell culture media. The concentration of VEGF in the cell culture media before treatment with BMP-2 was 11.2 pg/ml. The VEGF concentration after treatment with 0.500 pg/ml BMP-2 was 233.0 pg/ml and after treatment with 1 ng/ml BMP-2 was 2,969.0 pg/ml. The addition of increasing amounts of BMP-2 to lung A549 lung cancer cells growing in culture also caused a dose responsive increase in the expression of the oncogene Sonic Hedgehog. (Figure 12)

[00100] In addition, Applicants showed that BMP-2 stimulates the migration and invasion of the human lung cancer cell lines A549 and H7249. In one assay, recombinant BMP-2 caused a dose responsive increase in migration of cells from transwell migration chambers. (Figure 13(a)) In another, BMP-2 stimulated the migration of A549 and H7249 cells cultured on glass cover slips toward Affi-blue agarose beads containing recombinant BMP-2. (Figure 13 (c) and (d)) In addition, using transwell chambers coated with Matrigel, Applicants also showed that recombinant BMP-2 caused a dose responsive increase in the invasion of both A549 and H7249 cells. (Figure 13(e))

Effects of Inhibiting BMP-2 Expression

[00101] After finding that BMP-2 enhances cancer invasion and growth, Applicant conducted experiments to determine whether inhibitors of the activity of BMP-2 could be used to treat cancer. In these studies, recombinant mouse noggin (R & D Systems, Minneapolis, MN) was used as a representative inhibitor. Noggin, a natural inhibitor of BMP-2, is a secreted protein that binds BMP-2 and BMP-4, thereby preventing their activation of the BMP receptors. (Weaver, M., et al., Development 126: 4005-4115

(1999); Zimmerman, L.B., et al., Cell 86: 599-606 (1996); Tucker, A.S., et al., Science 282: 1136-1138 (1998); Capdevilla, J., et al., Developmental Biology 197: 205-217 (1998); Brunet, L.J., et al., Science 280: 1455-1447 (1998)) Mouse and human noggin are 98% homologous.

[00102] The effects of BMP-2 and noggin on tumor growth *in vivo* was examined by co-injecting the A549 cells subcutaneously into nude mice with Affi-Blue agarose beads coated with either albumin, recombinant human BMP-2, or recombinant human noggin. The animals were then sacrificed and tumors measured at 12 or 19 days. Inhibiting BMP-2 activity with noggin resulted in a statistically significant decrease in tumor growth. Addition of BMP-2 resulted in a statistically significant increase in tumor growth. (Figure 14)

[00103] Noggin also decreased the expression of VEGF and sonic hedgehog when added to A549 cells. (Figures 12 and 15)

[00104] Applicants also found that noggin completely inhibited the ability of BMP-2, discussed above, to enhance the migration of the A549 cells in a transwell chamber. (Figure 13(b))

EXAMPLES

Example 1: Identification of BMP-2 Using Representational Difference Analysis (RDA) Subtraction Technique

[00105] Representational difference analysis (RDA) subtraction technique was used to identify genes highly expressed in a non-small cell lung carcinoma obtained from a patient (tester) in comparison to normal bronchial human epithelial cells (driver). The technique for RDA described in the following references was followed: Holmes, M.L., et

al., Molecular and Cellular Biology 19: 4182-4190 (1999); Hubank, M., Nucleic Acids Research 22:5640-5648 (1994). Normal human bronchial epithelial cells were purchased from Clonetics, BioWhitaker (Walkersville, Maryland) and were maintained in serum free media. Human tissue specimens were obtained directly from the operating room and immediately frozen in liquid nitrogen. Tissue was stored in liquid nitrogen at –70C.

[00106] To perform RDA, mRNA was purified from the samples using Oligo dT columns (Pharmacia, Peapack, NJ) according to the manufacturer's instructions and cDNA was then obtained using the Pharmacia Time Saver cDNA synthesis kit also according to the manufacturer's instructions. cDNA was digested with Sau3A I endonuclease, R-linker ligated, and amplified by PCR. The R-linkers were removed and J-linkers ligated to the tester. The driver and tester cDNA were hybridized at 67 C for 20 hours (driver in excess 100:1) and the subtracted tester cDNA amplified by PCR. A second round of subtraction was performed using N-linkers (driver in excess 800,000:1). The amplified PCR products were cloned into blue script and sequenced using a IBI Prism 377 DNA sequencer. Known genes corresponding to the subtracted tumor cDNA were identified by a BLAST database search.

Example 2: Detection of Expression of BMP-2 in Human Lung Cancer Specimens Using RT-PCR

[00107] Reverse transcriptase polymerase chain reaction was performed using standard techniques well known in the art. The forward primer was acgagagctctcactggtcc (SEQ ID No: 15)The reverse primer was cattccggattacatgaggg (SEQ ID No: 16). The chain reaction consisted of denaturation at 95 C for 1 min, annealing at 54 C for 1 min, and extension at 72 C for 2 min with 33 cycles.

Example 3: Detection of Over-Expression of BMP and BMP Receptors in Various Cancer Tissue Specimens and Lung Cancer Cell Lines

[00108] Applicant detected expression of BMP and BMP receptors in a number of normal and cancerous tissue specimens and cells. As described above, all human tissue specimens were obtained directly from the operating room and immediately frozen in liquid nitrogen and stored at –70 C. Normal human bronchial epithelial (NBE) cells were purchased from Clonetics, BioWhitaker (Walkersville, Maryland) and were maintained in serum free media. Immortalized human bronchial epithelial (IHBE), BEAS-2B, cells were derived from normal bronchial epithelial cells immortalized with an adenovirus-12-5V40 hybrid virus (32). A549 and H7249 are highly invasive human lung cancer cell lines. The cell lines were cultured in 5% fetal bovine serum (FBS) in Dulbecco' s Modified Eagles medium (DME) containing penicillin, streptomycin, and glutamine with 5% pCO2 at 37^0C. Western blot analysis was used to detect expression of the BMP ligand and its receptors in all of these samples. Immunohistochemistry studies were performed to detect BMP in non-small cell lung carcinoma samples and normal lung tissue samples derived from patients.

Western Blot Analysis

[00109] In preparation for Western blot analysis, cells were lysed in a modified RIPA buffer containing 150 ml NaCl, 50 ml tris, pH 7.5, 1% NP 40, 10% deoxycholic acid, and protease inhibitor cocktail from Calbiochem. Tissue specimens were sonicated on ice in the same modified RIPA buffer. The protein concentration of the resulting samples was measured using the Bradford assay technique. Recombinant human BMP-2, purchased from R & D Systems and reconstituted in PBS with gelatin, served as a

control. Total cellular protein of the samples and recombinant human BMP-2 were analyzed by SDS-PAGE, transferred to nitrocellulose filter (Schleicher and Schuell, Keene, NH) at 35 V for 16 hours at 4oC and then incubated with the desired primary antibody. Specific proteins were detected using the enhanced chemiluminescence system (Amersham, Arlington Heights, IL).

[00110] The primary antibodies that were used included mouse anti-human BMP-2, goat anti-human BMP-4, goat anti-human BMP-7, goat anti-human type IA BMP receptor, and goat anti-human type IB BMP-2 receptor. All of these antibodies, except the goat anti-human BMP-7 were purchased from R & D Systems in Minneapolis, MN. The goat anti-human BMP-7 antibody was obtained from Santa Cruz (Santa Cruz, CA).

Immunohistochemistry Analysis

[0100] To perform immunohistochemistry analysis, four micron Cryostat-cut sections were air dried before being fixed in cold acetone for 10 minutes. Sections were washed in cold 0.5 M PBS and intrinsic peroxidase was quenched with 0.03% periodic acid for 20 minutes at room temperature. Sections were then rinsed in cold PBS and 0.5% BSA/PBS was applied to the slides for 15 minutes in a humid chamber. Biotinylated BMP-2/4 (R & D Systems) was applied at a 1:25 dilution in 1% BSA/PBS and incubated overnight at 4 C. Two slides were run as negative controls. One slide was incubated with biotinylated BMP-2 preabsorbed with recombinant human BMP-2 at 1:10 Molar ratio. As a second negative control slide samples were incubated overnight at 4 C with normal rabbit serum. Slides were washed with cold PBS and incubated for one hour in Streptavidin horseradish peroxidase (Dako) at a 1:500 dilution in 1% BSA/PBS. Slides were then counterstained in 0.7% Toluidine Blue.

Example 4: Detection of Processing of Mature BMP-2 by Human Leukocytes

[0101] Cell culture media from the A549 cells was incubated with leukocytes isolated from whole blood for 16 hours. Then, a Western blot was performed, as described above, on the cell culture media. Mouse anti-human BMP-2 antibody (#MAB355, R & D Systems, Minneapolis, MN) was the primary antibody used to detect the C-terminal end of BMP-2. Goat anti-human BMP-2 (Research Diagnostics, Flanders, NJ) was used to detect the N-terminal end of BMP-2. A Western blot of the leukocytes was also performed with an anti-furin primary antibody to determine that human leukocytes express furin convertase.

Example 5: Analysis of the Effect of BMP-2 and Noggin on Tumor Growth and Tumor Vasculature *In Vivo*

[0102] Nude mice studies were conducted to determine the effect of BMP-2 and one of its inhibitors, noggin, on tumor growth and tumor vasculature. 10^6 A549 cells were injected subcutaneously into nude mice with Affi-Blue agarose beads coated with albumin, recombinant human BMP-2 or recombinant mouse noggin. Both of these recombinant proteins were purchased from R & D Systems and were reconstituted in PBS with gelatin. Coating of Affi-Blue agarose beads with BMP-2 and noggin has been described in the literature. (Abe, E., et al., J. Bone Miner Res. 15: 663-673 (2000); Tucker, A.S., et al., Science 282: 1136-1138 (1998); Zimmerman, L.B., et al., Cell 86: 599-606 (1996)) In brief, 25 ug of Affi-blue agarose beads were incubated with 100 ug/ml albumin, recombinant human BMP-2, or recombinant noggin for 2 hours and then washed 3 times with PBS immediately prior to use. In separate experiments the beads

were not washed prior to injection. The coated beads were injected with the A549 cells into nude mice subcutaneously. To assess tumor growth after 12 or 19 days the length, width, and depth of the tumors were measured in mm. To assess tumor vasculature, tissue including a tumor was harvested after six days. Gross observations of the tissue were made. In addition, the tissue was stained with anti-CD 31 antibody, which recognizes endothelial cells. Vessels in five high power fields were counted by a person blinded to how the tumors were created.

Example 6: Effect of BMP-2 and Noggin on VEGF and Sonic Hedgehog Expression

Western blot analysis of VEGF and sonic hedgehog in presence of BMP-2 and noggin

[0103] Western blots, as described above, were performed on total cellular protein samples and cell culture media samples. The primary antibodies used to detect VEGF and sonic hedgehog were anti human VEGF from R & D Systems (Minneapolis, MN) and anti human sonic hedgehog from Santa Cruz (Santa Cruz, CA), respectively.

ELISA of VEGF in presence of BMP-2 and various concentrations of noggin

[0104] The sandwich ELISA method was used to determine VEGF concentrations in the cell culture media of A549 cells treated with noggin and in the cell culture media of human aortic endothelial cells treated with BMP-2. 100 ul of the monoclonal capture antibody, diluted in carbonate buffer (sodium bicarbonate, sodium carbonate, pH 9.0), was added to each well of a MaxiSorb Nunc-Immuno plate and incubated overnight at 4 C. The plates were washed two times with washing buffer (1x PBS with 0.0005% tween-20). Then, 200 ul of blocking buffer (1x PBS with 1% BSA) was added per well and incubated for 2 hours at room temperature. The plates were then washed 4 times with washing buffer.

[0105] The recombinant protein standards and samples (100ul per well) were added and the plate was then incubated overnight at 4 C. The plates were washed 5 times with washing buffer. The biotinylated detection antibody was diluted in incubation buffer (1x PBS with 10% fetal bovine serum) for a final concentration of 1 ug/ml. 100 ul of the detection antibody was added per well and incubated for 1 hour on a shaker at room temperature. The plates were washed 6 times with washing buffer and 100 ul of streptavidin-HRP conjugate (1:10,000) was added per well. The plates were incubated for 45 minutes at room temperature on a shaker and then washed 6 times with washing buffer. 100 ul/well of the substrate reagent (0.2 M citrate buffer, 1 mg/ml o-phenylenediamine dihydrocholoride (OPG), 3% hydrogen peroxide) was added and covered with aluminum foil for ten minutes. The reaction was stopped with 100 ul/well of 2M sulfuric acid and absorbance determined using an automated plate reader with a 490/690 filter. The protein concentration was then determined from the standard curve.

Example 7: Identification of BMP-2 as a Stimulant of Human Lung Cancer Cell Migration and Invasion

Migration Assay In Monolayer Culture

[0106] To detect BMP-induced migration in a monolayer culture, recombinant human BMP-2 (R & D systems, Minneapolis, MN) was coated to Affi-Blue agarose beads (Bio Rad, Hercules, CA) as described in the literature. (Vainio, S.; et al., Cell 75: 45-58 (1993); Sloan, A.J., et al., Arch Oral Biol. 44: 149-156 (1999)) Briefly, 100 ml of the Affi-Blue agarose beads were incubated with either 10 ml of recombinant BMP2 reconstituted in PBS with gelatin (100 mg/ml) or PBS alone at 37°C for 2 hours, washed with PBS, and reconstituted with 40 ml of PBS. Glass cover slips were coated with

serum free media containing BSA, fibronectin and collagen (32) and 50,000 cells were plated per cover slip in serum free media. Two microliters of the Affi-Blue agarose beads coated with recombinant BMP-2 or dilution buffer were placed in linear fashion next to the cover slips.

Chemotactic Assay

[0107] In the chemotactic assay, fifty thousand cells were placed in the upper chamber of an 8 micron transwell migration chamber (Becton Dickinson, Bedford, MA) and 300 ml of serum free media with 0 ng/ml, 1 ng/ml, 10 ng/ml, 100 ng/ml, 500 ng/ml, or 1000 ng/ml recombinant human BMP-2 placed in the lower well. After 24 hours the filters were then removed and the top of the filter wiped with a cotton swab and the cells that migrated through the filters were stained with Syto-16 intercalating dye. Five high power fields were counted using fluorescent microscopy. To show that noggin inhibits BMP-2 induced migration, the experiment was also performed with each of the following in the lower well of the transwell chamber: media alone, recombinant BMP-2 (500 ng/ml), and noggin (10 ug/ml) with recombinant BMP-2 (500 ng/ml).

Matrigel Invasion Assay

[0108] Invasion was studied using transwell chambers coated with 100 ml of Matrigel (Becton Dickinson). Fifty thousand cells were placed in the upper chamber and 300 ml of serum free media with 0ng/ml, 10 ng/ml, 100 ng/ml, 500 ng/ml, or 1000 ng/ml recombinant BMP-2 placed in the lower wells. After 48 hours the Matrigel was removed and cells that had migrated through the filter were stained with Syto-16 intercalating dye and 5 high power fields counted using fluorescent microscopy.

References

1. Thoracic Surgery (Churchill Livingstone, New York, Edinburgh, London, Melbourne, Tokyo) (1995).

2. Warburton, D., Schwarz, M., Tefft, D., Flores-Delgado, G., Anderson, K. D. & Cardoso, W. V. 2000) Mech Dev 92, 55-81.

3. Celeste AJ, I. J., Taylor RC, Hewick RM, Rosen V, Wang EA, Wozney JM (1990) Proc Natl Acad Sci USA 87, 9843-9847.

4. Erickson, D. M., Harris, S. E., Dean, D. D., Harris, M. A., Wozney, J. M., Boyan, B. D. & Schwartz, Z. (1997) J Orthop Res 15, 371-80.

5. An, J., Rosen, V., Cox, K., Beauchemin, N. & Sullivan, A. K. (1996) Exp Hematol 24, 768-75.

6. Abe, E., Yamamoto, M., Taguchi, Y., Lecka-Czemik, B., O'Brien, C. A., Economides, A. N., Stahl, N., Jilka, R. L. & Manolagas, S. C. (2000) J Bone Miner Res 15, 663-73.

7. Vainio, S., Karavanova, I., Jowett, A. & Thesleff, I. (1993) Cell 75, 45-58.

8. Weaver, M., Yingling, J. M., Dunn, N. R., Bellusci, S. & Hogan, B. L. (1999) Development 126, 4005-15.

9. Wozney, J. M., Rosen, V., Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W., Hewick, R. M. & Wang, E. A. (1988) Science 242, 1528-34.

10. Willette, R. N., Gu, J. L., Lysko, P. G., Anderson, K. M., Minehart, H. & Yue, T. (1999) J Vasc Res 36, 120-5.

11. Cunningham, N. S., Paralkar, V. & Reddi, A. H. (1992) Proc Natl Acad Sci U SA 89, 11740-4.

12. Cui, Y., Jean, F., Thomas, G. & Christian, J. L. (1998) Embo J 17, 4735-43.

13. Sugiura (1999) Biochem J 338, 443-440.

14. Liu, F., Ventura, F., Doody, J. & Massague, J. (1995) Mol Cell Biol 15, 3479-86.

15. Ikeda, T., Takahashi, H., Suzuki, A., Ueno, N., Yokose, S., Yamaguchi, A. & Yoshiki, 5. (1996) Dev Dyn 206, 3 18-29.

16. ten Dijke, P., Ichijo, H., Franzen, P., Schulz, P., Saras, J., Toyoshima, H., Heldin, C. H. & Miyazono, K. (1993) Oncogene 8, 2879-87.

17. ten Dijke, P., Yamashita, H., Sampath, T. K., Reddi, A. H., Estevez, M., Riddle, D. L., Ichijo, H., Heldin, C. H. & Miyazono, K. (1994) J Biol Chem 269, 16985-8.

18. Sakou, T., Onishi, T., Yamamoto, T., Nagamine, Sampath, T. & Ten Dijke, P. (1999) J Bone Miner Res 14, 1145-52.

SEQUENCE LISTING

<110> LANGENFELD, John

<120> BONE MORPHOGENETIC PROTEIN-2 IN THE TREATMENT AND DIAGNOSIS OF CANCER

<130> 270/070US

<140> to be assigned

<141> 2002-01-11

<150> US60/261,252

<151> 2001-01-12

<160> 16

<170> PatentIn version 3.1

<210> 1

<211> 1547

<212> DNA

<213> Homo sapiens

<220>

<221> source

<222> (1)..(1547)

<223> Homo sapiens: Taxon:9606

<220>
<221> gene
<222> (1)..(1547)
<223> BMP2

<220>
<221> CDS
<222> (324)..(1514)
<223>

<220>
<221> misc_feature
<222> (429)..(1127)
<223> Region: TGF-beta propeptide

<220>
<221> variation
<222> (432)..(432)
<223> Allele = "T"; Allele = "G"

<220>
<221> variation
<222> (584)..(584)
<223> Allele = "A"; Allele = "G"

<220>
<221> variation

<222> (893)..(893)

<223> Allele = "T"; Allele = "A"

<220>

<221> misc_feature

<222> (1209)..(1511)

<223> TGF-beta; Region: Transforming growth factor beta like domain

<220>

<221> misc_feature

<222> (1209)..(1511)

<223> TGFB; Region: Transforming growth factor-beta (TGF-beta) family

<400> 1

ggggacttct tgaacttgca gggagaataa cttgcgcacc ccactttgcg ccggtgcctt
60

tgccccagcg gagcctgctt cgccatctcc gagccccacc gcccctccac tcctcggcct
120

tgcccgacac tgagacgctg ttcccagcgt gaaaagagag actgcgcggc cggcacccgg
180

gagaaggagg aggcaaagaa aaggaacgga cattcggtcc ttgcgccagg tcctttgacc
240

agagtttttc catgtggacg ctctttcaat ggacgtgtcc ccgcgtgctt cttagacgga
300

ctgcggtctc ctaaaggtcg acc atg gtg gcc ggg acc cgc tgt ctt cta gcg
353
Met Val Ala Gly Thr Arg Cys Leu Leu Ala
1 5 10

ttg ctg ctt ccc cag gtc ctc ctg ggc ggc gcg gct ggc ctc gtt ccg
401
Leu Leu Leu Pro Gln Val Leu Leu Gly Gly Ala Ala Gly Leu Val Pro
15 20 25

gag ctg ggc cgc agg aag ttc gcg gcg gcg tcg tcg ggc cgc ccc tca
449

```
Glu Leu Gly Arg Arg Lys Phe Ala Ala Ala Ser Ser Gly Arg Pro Ser
            30                  35                  40

tcc cag ccc tct gac gag gtc ctg agc gag ttc gag ttg cgg ctg ctc
497
Ser Gln Pro Ser Asp Glu Val Leu Ser Glu Phe Glu Leu Arg Leu Leu
        45                  50                  55

agc atg ttc ggc ctg aaa cag aga ccc acc ccc agc agg gac gcc gtg
545
Ser Met Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser Arg Asp Ala Val
    60                  65                  70

gtg ccc ccc tac atg cta gac ctg tat cgc agg cac tca ggt cag ccg
593
Val Pro Pro Tyr Met Leu Asp Leu Tyr Arg Arg His Ser Gly Gln Pro
75                  80                  85                  90

ggc tca ccc gcc cca gac cac cgg ttg gag agg gca gcc agc cga gcc
641
Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala
                95                  100                 105

aac act gtg cgc agc ttc cac cat gaa gaa tct ttg gaa gaa cta cca
689
Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro
            110                 115                 120

gaa acg agt ggg aaa aca acc cgg aga ttc ttc ttt aat tta agt tct
737
Glu Thr Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser
        125                 130                 135

atc ccc acg gag gag ttt atc acc tca gca gag ctt cag gtt ttc cga
785
Ile Pro Thr Glu Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg
    140                 145                 150

gaa cag atg caa gat gct tta gga aac aat agc agt ttc cat cac cga
833
Glu Gln Met Gln Asp Ala Leu Gly Asn Asn Ser Ser Phe His His Arg
155                 160                 165                 170

att aat att tat gaa atc ata aaa cct gca aca gcc aac tcg aaa ttc
881
Ile Asn Ile Tyr Glu Ile Ile Lys Pro Ala Thr Ala Asn Ser Lys Phe
                175                 180                 185

ccc gtg acc aga ctt ttg gac acc agg ttg gtg aat cag aat gca agc
929
Pro Val Thr Arg Leu Leu Asp Thr Arg Leu Val Asn Gln Asn Ala Ser
            190                 195                 200

agg tgg gaa agt ttt gat gtc acc ccc gct gtg atg cgg tgg act gca
977
```

```
Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val Met Arg Trp Thr Ala
        205                 210                 215

cag gga cac gcc aac cat gga ttc gtg gtg gaa gtg gcc cac ttg gag
1025
Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His Leu Glu
    220                 225                 230

gag aaa caa ggt gtc tcc aag aga cat gtt agg ata agc agg tct ttg
1073
Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu
235                 240                 245                 250

cac caa gat gaa cac agc tgg tca cag ata agg cca ttg cta gta act
1121
His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr
                255                 260                 265

ttt ggc cat gat gga aaa ggg cat cct ctc cac aaa aga gaa aaa cgt
1169
Phe Gly His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg
            270                 275                 280

caa gcc aaa cac aaa cag cgg aaa cgc ctt aag tcc agc tgt aag aga
1217
Gln Ala Lys His Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg
        285                 290                 295

cac cct ttg tac gtg gac ttc agt gac gtg ggg tgg aat gac tgg att
1265
His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile
    300                 305                 310

gtg gct ccc ccg ggg tat cac gcc ttt tac tgc cac gga gaa tgc cct
1313
Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly Glu Cys Pro
315                 320                 325                 330

ttt cct ctg gct gat cat ctg aac tcc act aat cat gcc att gtt cag
1361
Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln
                335                 340                 345

acg ttg gtc aac tct gtt aac tct aag att cct aag gca tgc tgt gtc
1409
Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala Cys Cys Val
            350                 355                 360

ccg aca gaa ctc agt gct atc tcg atg ctg tac ctt gac gag aat gaa
1457
Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu
        365                 370                 375

aag gtt gta tta aag aac tat cag gac atg gtt gtg gag ggt tgt ggg
1505
```

Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu Gly Cys Gly
380 385 390

tgt cgc tag tacagcaaaa ttaaatacat aaatatatat ata
1547
Cys Arg
395

<210> 2

<211> 396

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<222> (429)..(1127)

<223> Region: TGF-beta propeptide

<220>

<221> misc_feature

<222> (1209)..(1511)

<223> TGF-beta; Region: Transforming growth factor beta like domain

<220>

<221> misc_feature

<222> (1209)..(1511)

<223> TGFB; Region: Transforming growth factor-beta (TGF-beta) family

<400> 2

Met Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val
1 5 10 15

Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys
20 25 30

Phe Ala Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu
35 40 45

```
Val Leu Ser Glu Phe Glu Leu Arg Leu Leu Ser Met Phe Gly Leu Lys
    50                  55                  60

Gln Arg Pro Thr Pro Ser Arg Asp Ala Val Val Pro Pro Tyr Met Leu
65                  70                  75                  80

Asp Leu Tyr Arg Arg His Ser Gly Gln Pro Gly Ser Pro Ala Pro Asp
                85                  90                  95

His Arg Leu Glu Arg Ala Ala Ser Arg Ala Asn Thr Val Arg Ser Phe
            100                 105                 110

His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr Ser Gly Lys Thr
        115                 120                 125

Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu Glu Phe
    130                 135                 140

Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln Met Gln Asp Ala
145                 150                 155                 160

Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile
                165                 170                 175

Ile Lys Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu
            180                 185                 190

Asp Thr Arg Leu Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp
        195                 200                 205

Val Thr Pro Ala Val Met Arg Trp Thr Ala Gln Gly His Ala Asn His
    210                 215                 220

Gly Phe Val Val Glu Val Ala His Leu Glu Glu Lys Gln Gly Val Ser
225                 230                 235                 240

Lys Arg His Val Arg Ile Ser Arg Ser Leu His Gln Asp Glu His Ser
                245                 250                 255

Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp Gly Lys
```

260 265 270

Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His Lys Gln
275 280 285

Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
290 295 300

Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr
305 310 315 320

His Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His
325 330 335

Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val
340 345 350

Asn Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala
355 360 365

Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn
370 375 380

Tyr Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg
385 390 395

<210> 3

<211> 699

<212> DNA

<213> Homo sapiens

<220>

<221> source

<222> (1)..(699)

<223> Homo sapiens: Taxon:9606

<220>

<221> gene

<222> (1)..(699)

<223> NOG

<220>

<221> sig_peptide

<222> (1)..(57)

<223>

<220>

<221> CDS

<222> (1)..(699)

<223> Noggin, mouse, homolog of

<300>

<301> Valenzuela,D.M., Economides,A.N., Rojas,E., Lamb,T.M., Nunez,L., Jones,P., Ip,N.Y., Espinosa,R., Brannan,C.I., Gilbert,D.J., Copeland,N.G., Jenkins,N.A., LeBeau,M.M., Harland,R.M. and Yancopoulos,G.D.

<302> Identification of mammalian noggin and its expression in the adult nervous system

<303> J. Neurosci.

<304> 15

<305> 9

<306> 6077-6084

<307> 1995

<308> NM_005450

<309> 2000-11-01

<313> (1)..(699)

<300>

<301> McMahon,J.A., Takada,S., Zimmerman,L.B., Fan,C.M., Harland,R.M. and McMahon, A.P.

<302> Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite

<303> Genes Dev.

<304> 12

<305> 10

<306> 1438-1452

<307> 1998

<308> NM_005450

<309> 2000-11-01

<313> (1)..(699)

<300>

<301> Brunet,L.J., McMahon,J.A., McMahon,A.P. and Harland,R.M.

<302> Noggin, cartilage morphogenesis, and joint formation in the mammalian skeleton

<303> Science

<304> 280

<305> 5368

<306> 1455-1457

<307> 1998

<308> NM_005450

<309> 2000-11-01

<313> (1)..(699)

<300>

<301> Smith, W.C.

<302> TGF beta inhibitors. New and unexpected requirements in vertebrate development

<303> Trends Genet.

<304> 15

<305> 1

<306> 3-5

<307> 1999

<308> NM_005450

<309> 2000-11-01

<313> (1)..(699)

<300>

<301> Gong,Y., Krakow,D., Marcelino,J., Wilkin,D., Chitayat,D., Babul-Hirji,R., Hudgins,L., Cremers,C.W., Cremers,F.P., Brunner,H.G., Reinker,K., Rimoin,D.L., Cohn,D.H., Goodman,F.R., Reardon,W., Patton,M., Francomano,C.A. and Warman,M.L.

<302> Heterozygous mutations in the gene encoding noggin affect human joint morphogenesis

<303> Nat. Genet.

<304> 21

<305> 3

<306> 302-304

<307> 1999

<308> NM_005450

<309> 2000-11-01

<313> (1)..(699)

<400> 3
atg gag cgc tgc ccc agc cta ggg gtc acc ctc tac gcc ctg gtg gtg
48

```
Met Glu Arg Cys Pro Ser Leu Gly Val Thr Leu Tyr Ala Leu Val Val
1               5                  10                  15

gtc ctg ggg ctg cgg gcg aca ccg gcc ggc ggc cag cac tat ctc cac
96
Val Leu Gly Leu Arg Ala Thr Pro Ala Gly Gly Gln His Tyr Leu His
            20                  25                  30

atc cgc ccg gca ccc agc gac aac ctg ccc ctg gtg gac ctc atc gaa
144
Ile Arg Pro Ala Pro Ser Asp Asn Leu Pro Leu Val Asp Leu Ile Glu
        35                  40                  45

cac cca gac cct atc ttt gac ccc aag gaa aag gat ctg aac gag acg
192
His Pro Asp Pro Ile Phe Asp Pro Lys Glu Lys Asp Leu Asn Glu Thr
    50                  55                  60

ctg ctg cgc tcg ctg ctc ggg ggc cac tac gac cca ggc ttc atg gcc
240
Leu Leu Arg Ser Leu Leu Gly Gly His Tyr Asp Pro Gly Phe Met Ala
65                  70                  75                  80

acc tcg ccc ccc gag gac cgg ccc ggc ggg ggc ggg ggt gca gct ggg
288
Thr Ser Pro Pro Glu Asp Arg Pro Gly Gly Gly Gly Gly Ala Ala Gly
                85                  90                  95

ggc gcg gag gac ctg gcg gag ctg gac cag ctg ctg cgg cag cgg ccg
336
Gly Ala Glu Asp Leu Ala Glu Leu Asp Gln Leu Leu Arg Gln Arg Pro
            100                 105                 110

tcg ggg gcc atg ccg agc gag atc aaa ggg cta gag ttc tcc gag ggc
384
Ser Gly Ala Met Pro Ser Glu Ile Lys Gly Leu Glu Phe Ser Glu Gly
        115                 120                 125

ttg gcc cag ggc aag aag cag cgc cta agc aag aag ctg cgg agg aag
432
Leu Ala Gln Gly Lys Lys Gln Arg Leu Ser Lys Lys Leu Arg Arg Lys
    130                 135                 140

tta cag atg tgg ctg tgg tcg cag aca ttc tgc ccc gtg ctg tac gcg
480
Leu Gln Met Trp Leu Trp Ser Gln Thr Phe Cys Pro Val Leu Tyr Ala
145                 150                 155                 160

tgg aac gac ctg ggc agc cgc ttt tgg ccg cgc tac gtg aag gtg ggc
528
Trp Asn Asp Leu Gly Ser Arg Phe Trp Pro Arg Tyr Val Lys Val Gly
                165                 170                 175

agc tgc ttc agt aag cgc tcg tgc tcc gtg ccc gag ggc atg gtg tgc
576
```

```
Ser Cys Phe Ser Lys Arg Ser Cys Ser Val Pro Glu Gly Met Val Cys
            180                 185                 190

aag ccg tcc aag tcc gtg cac ctc acg gtg ctg cgg tgg cgc tgt cag
624
Lys Pro Ser Lys Ser Val His Leu Thr Val Leu Arg Trp Arg Cys Gln
        195                 200                 205

cgg cgc ggg ggc cag cgc tgc ggc tgg att ccc atc cag tac ccc atc
672
Arg Arg Gly Gly Gln Arg Cys Gly Trp Ile Pro Ile Gln Tyr Pro Ile
    210                 215                 220

att tcc gag tgc aag tgc tcg tgc tag
699
Ile Ser Glu Cys Lys Cys Ser Cys
225                 230
```

<210> 4

<211> 232

<212> PRT

<213> Homo sapiens

<400> 4

```
Met Glu Arg Cys Pro Ser Leu Gly Val Thr Leu Tyr Ala Leu Val Val
1               5                   10                  15

Val Leu Gly Leu Arg Ala Thr Pro Ala Gly Gly Gln His Tyr Leu His
            20                  25                  30

Ile Arg Pro Ala Pro Ser Asp Asn Leu Pro Leu Val Asp Leu Ile Glu
        35                  40                  45

His Pro Asp Pro Ile Phe Asp Pro Lys Glu Lys Asp Leu Asn Glu Thr
    50                  55                  60

Leu Leu Arg Ser Leu Leu Gly Gly His Tyr Asp Pro Gly Phe Met Ala
65                  70                  75                  80

Thr Ser Pro Pro Glu Asp Arg Pro Gly Gly Gly Gly Gly Ala Ala Gly
                85                  90                  95
```

Gly Ala Glu Asp Leu Ala Glu Leu Asp Gln Leu Leu Arg Gln Arg Pro
100 105 110

Ser Gly Ala Met Pro Ser Glu Ile Lys Gly Leu Glu Phe Ser Glu Gly
115 120 125

Leu Ala Gln Gly Lys Lys Gln Arg Leu Ser Lys Lys Leu Arg Arg Lys
130 135 140

Leu Gln Met Trp Leu Trp Ser Gln Thr Phe Cys Pro Val Leu Tyr Ala
145 150 155 160

Trp Asn Asp Leu Gly Ser Arg Phe Trp Pro Arg Tyr Val Lys Val Gly
165 170 175

Ser Cys Phe Ser Lys Arg Ser Cys Ser Val Pro Glu Gly Met Val Cys
180 185 190

Lys Pro Ser Lys Ser Val His Leu Thr Val Leu Arg Trp Arg Cys Gln
195 200 205

Arg Arg Gly Gly Gln Arg Cys Gly Trp Ile Pro Ile Gln Tyr Pro Ile
210 215 220

Ile Ser Glu Cys Lys Cys Ser Cys
225 230

<210> 5

<211> 699

<212> DNA

<213> Mus musculus

<220>

<221> gene

<222> (1)..(699)

<223> nog

<220>

<221> CDS

<222> (1)..(699)

<223> nog

<400> 5

atg gag cgc tgc ccc agc ctg ggg gtc acc ctc tac gcc ctg gtg gtg
48
Met Glu Arg Cys Pro Ser Leu Gly Val Thr Leu Tyr Ala Leu Val Val
1 5 10 15

gtc ctg ggg ctg cgg gca gca cca gcc ggc ggc cag cac tat cta cac
96
Val Leu Gly Leu Arg Ala Ala Pro Ala Gly Gly Gln His Tyr Leu His
20 25 30

atc cgc cca gca ccc agc gac aac ctg ccc ttg gtg gac ctc atc gaa
144
Ile Arg Pro Ala Pro Ser Asp Asn Leu Pro Leu Val Asp Leu Ile Glu
35 40 45

cat cca gac cct atc ttt gac cct aag gag aag gat ctg aac gag acg
192
His Pro Asp Pro Ile Phe Asp Pro Lys Glu Lys Asp Leu Asn Glu Thr
50 55 60

ctg ctg cgc tcg ctg ctc ggg ggc cac tac gac ccg ggc ttt atg gcc
240
Leu Leu Arg Ser Leu Leu Gly Gly His Tyr Asp Pro Gly Phe Met Ala
65 70 75 80

act tcg ccc cca gag gac cga ccc gga ggg ggc ggg gga ccg gct gga
288
Thr Ser Pro Pro Glu Asp Arg Pro Gly Gly Gly Gly Gly Pro Ala Gly
85 90 95

ggt gcc gag gac ctg gcg gag ctg gac cag ctg ctg cgg cag cgg ccg
336
Gly Ala Glu Asp Leu Ala Glu Leu Asp Gln Leu Leu Arg Gln Arg Pro
100 105 110

tcg ggg gcc atg ccg agc gag atc aaa ggg ctg gag ttc tcc gag ggc
384
Ser Gly Ala Met Pro Ser Glu Ile Lys Gly Leu Glu Phe Ser Glu Gly
115 120 125

ttg gcc caa ggc aag aaa cag cgc ctg agc aag aag ctg agg agg aag
432
Leu Ala Gln Gly Lys Lys Gln Arg Leu Ser Lys Lys Leu Arg Arg Lys

```
    130                 135                 140

tta cag atg tgg ctg tgg tca cag acc ttc tgc ccg gtg ctg tac gcg
480
Leu Gln Met Trp Leu Trp Ser Gln Thr Phe Cys Pro Val Leu Tyr Ala
145                 150                 155                 160

tgg aat gac cta ggc agc cgc ttt tgg cca cgc tac gtg aag gtg ggc
528
Trp Asn Asp Leu Gly Ser Arg Phe Trp Pro Arg Tyr Val Lys Val Gly
                165                 170                 175

agc tgc ttc agc aag cgc tcc tgc tct gtg ccc gag ggc atg gtg tgt
576
Ser Cys Phe Ser Lys Arg Ser Cys Ser Val Pro Glu Gly Met Val Cys
            180                 185                 190

aag cca tcc aag tct gtg cac ctc acg gtg ctg cgg tgg cgc tgt cag
624
Lys Pro Ser Lys Ser Val His Leu Thr Val Leu Arg Trp Arg Cys Gln
        195                 200                 205

cgg cgc ggg ggt cag cgc tgc ggc tgg att ccc atc cag tac ccc atc
672
Arg Arg Gly Gly Gln Arg Cys Gly Trp Ile Pro Ile Gln Tyr Pro Ile
    210                 215                 220

att tcc gag tgt aag tgt tcc tgc tag
699
Ile Ser Glu Cys Lys Cys Ser Cys
225                 230
```

<210> 6

<211> 232

<212> PRT

<213> Mus musculus

<400> 6

```
Met Glu Arg Cys Pro Ser Leu Gly Val Thr Leu Tyr Ala Leu Val Val
1               5                   10                  15

Val Leu Gly Leu Arg Ala Ala Pro Ala Gly Gly Gln His Tyr Leu His
            20                  25                  30

Ile Arg Pro Ala Pro Ser Asp Asn Leu Pro Leu Val Asp Leu Ile Glu
        35                  40                  45
```

His Pro Asp Pro Ile Phe Asp Pro Lys Glu Lys Asp Leu Asn Glu Thr
50 55 60

Leu Leu Arg Ser Leu Leu Gly Gly His Tyr Asp Pro Gly Phe Met Ala
65 70 75 80

Thr Ser Pro Pro Glu Asp Arg Pro Gly Gly Gly Gly Gly Pro Ala Gly
85 90 95

Gly Ala Glu Asp Leu Ala Glu Leu Asp Gln Leu Leu Arg Gln Arg Pro
100 105 110

Ser Gly Ala Met Pro Ser Glu Ile Lys Gly Leu Glu Phe Ser Glu Gly
115 120 125

Leu Ala Gln Gly Lys Lys Gln Arg Leu Ser Lys Lys Leu Arg Arg Lys
130 135 140

Leu Gln Met Trp Leu Trp Ser Gln Thr Phe Cys Pro Val Leu Tyr Ala
145 150 155 160

Trp Asn Asp Leu Gly Ser Arg Phe Trp Pro Arg Tyr Val Lys Val Gly
165 170 175

Ser Cys Phe Ser Lys Arg Ser Cys Ser Val Pro Glu Gly Met Val Cys
180 185 190

Lys Pro Ser Lys Ser Val His Leu Thr Val Leu Arg Trp Arg Cys Gln
195 200 205

Arg Arg Gly Gly Gln Arg Cys Gly Trp Ile Pro Ile Gln Tyr Pro Ile
210 215 220

Ile Ser Glu Cys Lys Cys Ser Cys
225 230

<210> 7

<211> 3547

<212> DNA

<213> Homo sapiens

<220>

<221> source

<222> (1)..(3547)

<223> Taxon:9606

<220>

<221> gene

<222> (1)..(3547)

<223> CHRD

<220>

<221> CDS

<222> (247)..(3114)

<223> Alternatively spliced

<300>

<301> Millet, C., Lemaire, P., Orsetti, B., Guglielmi, P., and Francois, V.

<302> The human chordin gene encodes several differentially expressed spliced variants with distinct BMP opposing activities

<303> Mech. Dev.

<304> 106

<305> 1

<306> 85-96

<307> 2001

<308> AF209928

<309> 2001-08-03

<313> (1)..(3547)

<300>

<301> Millet, C., and Francois, V.

<302> Direct Submission

<303> Institut de Genetique Humaine

<304> 1

<305> 1

<306> 1-2

<307> 1999-11-30

<308> AF209928

<309> 2001-08-03

<313> (1)..(3547)

<400> 7

cccgggtcag cgcccgcccg cccgcgctcc tcccggccgc tcctcccgcc ccgcccggcc 60

cggcgccgac tctgcggccg cccgacgagc ccctcgcggc actgccccgg ccccggcccc 120

ggccccggcc ccctcccgcc gcaccgcccc cggcccggcc ctccgccctc cgcactcccg 180

cctccctccc tccgcccgct cccgcgccct cctccctccc tcctccccag ctgtcccgtt 240

cgcgtc atg ccg agc ctc ccg gcc ccg ccg gcc ccg ctg ctg ctc ctc 288
Met Pro Ser Leu Pro Ala Pro Pro Ala Pro Leu Leu Leu Leu
1 5 10

ggg ctg ctg ctg ctc ggc tcc cgg ccg gcc cgc ggc gcc ggc ccc gag 336
Gly Leu Leu Leu Leu Gly Ser Arg Pro Ala Arg Gly Ala Gly Pro Glu
15 20 25 30

ccc ccc gtg ctg ccc atc cgt tct gag aag gag ccg ctg ccc gtt cgg 384

```
Pro Pro Val Leu Pro Ile Arg Ser Glu Lys Glu Pro Leu Pro Val Arg
                35                  40                  45

gga gcg gca ggc tgc acc ttc ggc ggg aag gtc tat gcc ttg gac gag
432
Gly Ala Ala Gly Cys Thr Phe Gly Gly Lys Val Tyr Ala Leu Asp Glu
            50                  55                  60

acg tgg cac ccg gac cta ggg gag cca ttc ggg gtg atg cgc tgc gtg
480
Thr Trp His Pro Asp Leu Gly Glu Pro Phe Gly Val Met Arg Cys Val
        65                  70                  75

ctg tgc gcc tgc gag gcg cct cag tgg ggt cgc cgt acc agg ggc cct
528
Leu Cys Ala Cys Glu Ala Pro Gln Trp Gly Arg Arg Thr Arg Gly Pro
    80                  85                  90

ggc agg gtc agc tgc aag aac atc aaa cca gag tgc cca acc ccg gcc
576
Gly Arg Val Ser Cys Lys Asn Ile Lys Pro Glu Cys Pro Thr Pro Ala
95                  100                 105                 110

tgt ggg cag ccg cgc cag ctg ccg gga cac tgc tgc cag acc tgc ccc
624
Cys Gly Gln Pro Arg Gln Leu Pro Gly His Cys Cys Gln Thr Cys Pro
                115                 120                 125

cag gag cgc agc agt tcg gag cgg cag ccg agc ggc ctg tcc ttc gag
672
Gln Glu Arg Ser Ser Ser Glu Arg Gln Pro Ser Gly Leu Ser Phe Glu
            130                 135                 140

tat ccg cgg gac ccg gag cat cgc agt tat agc gac cgc ggg gag cca
720
Tyr Pro Arg Asp Pro Glu His Arg Ser Tyr Ser Asp Arg Gly Glu Pro
        145                 150                 155

ggc gct gag gag cgg gcc cgt ggt gac ggc cac acg gac ttc gtg gcg
768
Gly Ala Glu Glu Arg Ala Arg Gly Asp Gly His Thr Asp Phe Val Ala
    160                 165                 170

ctg ctg aca ggg ccg agg tcg cag gcg gtg gca cga gcc cga gtc tcg
816
Leu Leu Thr Gly Pro Arg Ser Gln Ala Val Ala Arg Ala Arg Val Ser
175                 180                 185                 190

ctg ctg cgc tct agc ctc cgc ttc tct atc tcc tac agg cgg ctg gac
864
Leu Leu Arg Ser Ser Leu Arg Phe Ser Ile Ser Tyr Arg Arg Leu Asp
                195                 200                 205

cgc cct acc agg atc cgc ttc tca gac tcc aat ggc agt gtc ctg ttt
912
```

```
Arg Pro Thr Arg Ile Arg Phe Ser Asp Ser Asn Gly Ser Val Leu Phe
            210                 215                 220

gag cac cct gca gcc ccc acc caa gat ggc ctg gtc tgt ggg gtg tgg
960
Glu His Pro Ala Ala Pro Thr Gln Asp Gly Leu Val Cys Gly Val Trp
        225                 230                 235

cgg gca gtg cct cgg ttg tct ctg cgg ctc ctt agg gca gaa cag ctg
1008
Arg Ala Val Pro Arg Leu Ser Leu Arg Leu Leu Arg Ala Glu Gln Leu
    240                 245                 250

cat gtg gca ctt gtg aca ctc act cac cct tca ggg gag gtc tgg ggg
1056
His Val Ala Leu Val Thr Leu Thr His Pro Ser Gly Glu Val Trp Gly
255                 260                 265                 270

cct ctc atc cgg cac cgg gcc ctg gct gca gag acc ttc agt gcc atc
1104
Pro Leu Ile Arg His Arg Ala Leu Ala Ala Glu Thr Phe Ser Ala Ile
                275                 280                 285

ctg act cta gaa ggc ccc cca cag cag ggc gta ggg ggc atc acc ctg
1152
Leu Thr Leu Glu Gly Pro Pro Gln Gln Gly Val Gly Gly Ile Thr Leu
            290                 295                 300

ctc act ctc agt gac aca gag gac tcc ttg cat ttt ttg ctg ctc ttc
1200
Leu Thr Leu Ser Asp Thr Glu Asp Ser Leu His Phe Leu Leu Leu Phe
        305                 310                 315

cga ggg ctg ctg gaa ccc agg agt ggg gga cta acc cag gtt ccc ttg
1248
Arg Gly Leu Leu Glu Pro Arg Ser Gly Gly Leu Thr Gln Val Pro Leu
    320                 325                 330

agg ctc cag att cta cac cag ggg cag cta ctg cga gaa ctt cag gcc
1296
Arg Leu Gln Ile Leu His Gln Gly Gln Leu Leu Arg Glu Leu Gln Ala
335                 340                 345                 350

aat gtc tca gcc cag gaa cca ggc ttt gct gag gtg ctg ccc aac ctg
1344
Asn Val Ser Ala Gln Glu Pro Gly Phe Ala Glu Val Leu Pro Asn Leu
                355                 360                 365

aca gtc cag gag atg gac tgg ctg gtg ctg ggg gag ctg cag atg gcc
1392
Thr Val Gln Glu Met Asp Trp Leu Val Leu Gly Glu Leu Gln Met Ala
            370                 375                 380

ctg gag tgg gca ggc agg cca ggg ctg cgc atc agt gga cac att gct
1440
```

```
Leu Glu Trp Ala Gly Arg Pro Gly Leu Arg Ile Ser Gly His Ile Ala
        385                 390                 395

gcc agg aag agc tgc gac gtc ctg caa agt gtc ctt tgt ggg gct gat
1488
Ala Arg Lys Ser Cys Asp Val Leu Gln Ser Val Leu Cys Gly Ala Asp
    400                 405                 410

gcc ctg atc cca gtc cag acg ggt gct gcc ggc tca gcc agc ctc acg
1536
Ala Leu Ile Pro Val Gln Thr Gly Ala Ala Gly Ser Ala Ser Leu Thr
415                 420                 425                 430

ctg cta gga aat ggc tcc ctg atc tat cag gtg caa gtg gta ggg aca
1584
Leu Leu Gly Asn Gly Ser Leu Ile Tyr Gln Val Gln Val Val Gly Thr
                435                 440                 445

agc agt gag gtg gtg gcc atg aca ctg gag acc aag cct cag cgg agg
1632
Ser Ser Glu Val Val Ala Met Thr Leu Glu Thr Lys Pro Gln Arg Arg
            450                 455                 460

gat cag cgc act gtc ctg tgc cac atg gct gga ctc cag cca gga gga
1680
Asp Gln Arg Thr Val Leu Cys His Met Ala Gly Leu Gln Pro Gly Gly
        465                 470                 475

cac acg gcc gtg ggt atc tgc cct ggg ctg ggt gcc cga ggg gct cat
1728
His Thr Ala Val Gly Ile Cys Pro Gly Leu Gly Ala Arg Gly Ala His
    480                 485                 490

atg ctg ctg cag aat gag ctc ttc ctg aat gtg ggc acc aag gac ttc
1776
Met Leu Leu Gln Asn Glu Leu Phe Leu Asn Val Gly Thr Lys Asp Phe
495                 500                 505                 510

cca gac gga gag ctt cgg ggg cac gtg gct gcc ctg ccc tac tgt ggg
1824
Pro Asp Gly Glu Leu Arg Gly His Val Ala Ala Leu Pro Tyr Cys Gly
                515                 520                 525

cat agc gcc cgc cat gac acg ctg ccc gtg ccc cta gca gga gcc ctg
1872
His Ser Ala Arg His Asp Thr Leu Pro Val Pro Leu Ala Gly Ala Leu
            530                 535                 540

gtg cta ccc cct gtg aag agc caa gca gca ggg cac gcc tgg ctt tcc
1920
Val Leu Pro Pro Val Lys Ser Gln Ala Ala Gly His Ala Trp Leu Ser
        545                 550                 555

ttg gat acc cac tgt cac ctg cac tat gaa gtg ctg ctg gct ggg ctt
1968
```

```
Leu Asp Thr His Cys His Leu His Tyr Glu Val Leu Leu Ala Gly Leu
    560                 565                 570

ggt ggc tca gaa caa ggc act gtc act gcc cac ctc ctt ggg cct cct
2016
Gly Gly Ser Glu Gln Gly Thr Val Thr Ala His Leu Leu Gly Pro Pro
575                 580                 585                 590

gga acg cca ggg cct cgg cgg ctg ctg aag gga ttc tat ggc tca gag
2064
Gly Thr Pro Gly Pro Arg Arg Leu Leu Lys Gly Phe Tyr Gly Ser Glu
                595                 600                 605

gcc cag ggt gtg gtg aag gac ctg gag ccg gaa ctg ctg cgg cac ctg
2112
Ala Gln Gly Val Val Lys Asp Leu Glu Pro Glu Leu Leu Arg His Leu
            610                 615                 620

gca aaa ggc atg gcc tcc ctg ctg atc acc acc aag ggt agc ccc aga
2160
Ala Lys Gly Met Ala Ser Leu Leu Ile Thr Thr Lys Gly Ser Pro Arg
        625                 630                 635

ggg gag ctc cga ggg cag gtg cac ata gcc aac caa tgt gag gtt ggc
2208
Gly Glu Leu Arg Gly Gln Val His Ile Ala Asn Gln Cys Glu Val Gly
    640                 645                 650

gga ctg cgc ctg gag gcg gcc ggg gcc gag ggg gtg cgg gcg ctg ggg
2256
Gly Leu Arg Leu Glu Ala Ala Gly Ala Glu Gly Val Arg Ala Leu Gly
655                 660                 665                 670

gct ccg gat aca gcc tct gct gcg ccg cct gtg gtg cct ggt ctc ccg
2304
Ala Pro Asp Thr Ala Ser Ala Ala Pro Pro Val Val Pro Gly Leu Pro
                675                 680                 685

gcc cta gcg ccc gcc aaa cct ggt ggt cct ggg cgg ccc cga gac ccc
2352
Ala Leu Ala Pro Ala Lys Pro Gly Gly Pro Gly Arg Pro Arg Asp Pro
            690                 695                 700

aac aca tgc ttc ttc gag ggg cag cag cgc ccc cac ggg gct cgc tgg
2400
Asn Thr Cys Phe Phe Glu Gly Gln Gln Arg Pro His Gly Ala Arg Trp
        705                 710                 715

gcg ccc aac tac gac ccg ctc tgc tca ctc tgc acc tgc cag aga cga
2448
Ala Pro Asn Tyr Asp Pro Leu Cys Ser Leu Cys Thr Cys Gln Arg Arg
    720                 725                 730

acg gtg atc tgt gac ccg gtg gtg tgc cca ccg ccc agc tgc cca cac
2496
```

```
Thr Val Ile Cys Asp Pro Val Val Cys Pro Pro Pro Ser Cys Pro His
735                 740                 745                 750

ccg gtg cag gct ccc gac cag tgc tgc cct gtt tgc cct gag aaa caa
2544
Pro Val Gln Ala Pro Asp Gln Cys Cys Pro Val Cys Pro Glu Lys Gln
                755                 760                 765

gat gtc aga gac ttg cca ggg ctg cca agg agc cgg gac cca gga gag
2592
Asp Val Arg Asp Leu Pro Gly Leu Pro Arg Ser Arg Asp Pro Gly Glu
            770                 775                 780

ggc tgc tat ttt gat ggt gac cgg agc tgg cgg gca gcg ggt acg cgg
2640
Gly Cys Tyr Phe Asp Gly Asp Arg Ser Trp Arg Ala Ala Gly Thr Arg
        785                 790                 795

tgg cac ccc gtt gtg ccc ccc ttt ggc tta att aag tgt gct gtc tgc
2688
Trp His Pro Val Val Pro Pro Phe Gly Leu Ile Lys Cys Ala Val Cys
    800                 805                 810

acc tgc aag ggg ggc act gga gag gtg cac tgt gag aag gtg cag tgt
2736
Thr Cys Lys Gly Gly Thr Gly Glu Val His Cys Glu Lys Val Gln Cys
815                 820                 825                 830

ccc cgg ctg gcc tgt gcc cag cct gtg cgt gtc aac ccc acc gac tgc
2784
Pro Arg Leu Ala Cys Ala Gln Pro Val Arg Val Asn Pro Thr Asp Cys
                835                 840                 845

tgc aaa cag tgt cca gtg ggg tcg ggg gcc cac ccc cag ctg ggg gac
2832
Cys Lys Gln Cys Pro Val Gly Ser Gly Ala His Pro Gln Leu Gly Asp
            850                 855                 860

ccc atg cag gct gat ggg ccc cgg ggc tgc cgt ttt gct ggg cag tgg
2880
Pro Met Gln Ala Asp Gly Pro Arg Gly Cys Arg Phe Ala Gly Gln Trp
        865                 870                 875

ttc cca gag agt cag agc tgg cac ccc tca gtg ccc cct ttt gga gag
2928
Phe Pro Glu Ser Gln Ser Trp His Pro Ser Val Pro Pro Phe Gly Glu
    880                 885                 890

atg agc tgt atc acc tgc aga tgt ggg gca ggg gtg cct cac tgt gag
2976
Met Ser Cys Ile Thr Cys Arg Cys Gly Ala Gly Val Pro His Cys Glu
895                 900                 905                 910

cgg gat gac tgt tca ctg cca ctg tcc tgt ggc tcg ggg aag gag agt
3024
```

Arg Asp Asp Cys Ser Leu Pro Leu Ser Cys Gly Ser Gly Lys Glu Ser
915 920 925

cga tgc tgt tcc cgc tgc acg gcc cac cgg cgg cca gcc cca gag acc
3072
Arg Cys Cys Ser Arg Cys Thr Ala His Arg Arg Pro Ala Pro Glu Thr
930 935 940

aga act gat cca gag ctg gag aaa gaa gcc gaa ggc tct tag
3114
Arg Thr Asp Pro Glu Leu Glu Lys Glu Ala Glu Gly Ser
945 950 955

ggagcagcca gagggccaag tgaccaagag gatggggcct gagctgggga aggggtggca
3174

tcgaggacct tcttgcattc tcctgtggga agcccagtgc ctttgctcct ctgtcctgcc
3234

tctactccca cccccactac ctctgggaac cacagctcca caagggggag aggcagctgg
3294

gccagaccga ggtcacagcc actccaagtc ctgccctgcc accctcggcc tctgtcctgg
3354

aagccccacc cctttcctcc tgtacataat gtcactggct tgttgggatt tttaatttat
3414

cttcactcag caccaagggc ccccgacact ccactcctgc tgcccctgag ctgagcagag
3474

tcattattgg agagttttgt atttattaaa acatttcttt ttcagtcaaa aaaaaaaaaa
3534

aaaaaaaaaa aaa
3547

<210> 8

<211> 955

<212> PRT

<213> Homo sapiens

<400> 8

Met Pro Ser Leu Pro Ala Pro Pro Ala Pro Leu Leu Leu Leu Gly Leu
1 5 10 15

Leu Leu Leu Gly Ser Arg Pro Ala Arg Gly Ala Gly Pro Glu Pro Pro

```
                20                  25                  30

Val Leu Pro Ile Arg Ser Glu Lys Glu Pro Leu Pro Val Arg Gly Ala
        35                  40                  45

Ala Gly Cys Thr Phe Gly Gly Lys Val Tyr Ala Leu Asp Glu Thr Trp
    50                  55                  60

His Pro Asp Leu Gly Glu Pro Phe Gly Val Met Arg Cys Val Leu Cys
65                  70                  75                  80

Ala Cys Glu Ala Pro Gln Trp Gly Arg Arg Thr Arg Gly Pro Gly Arg
                85                  90                  95

Val Ser Cys Lys Asn Ile Lys Pro Glu Cys Pro Thr Pro Ala Cys Gly
            100                 105                 110

Gln Pro Arg Gln Leu Pro Gly His Cys Cys Gln Thr Cys Pro Gln Glu
        115                 120                 125

Arg Ser Ser Ser Glu Arg Gln Pro Ser Gly Leu Ser Phe Glu Tyr Pro
    130                 135                 140

Arg Asp Pro Glu His Arg Ser Tyr Ser Asp Arg Gly Glu Pro Gly Ala
145                 150                 155                 160

Glu Glu Arg Ala Arg Gly Asp Gly His Thr Asp Phe Val Ala Leu Leu
                165                 170                 175

Thr Gly Pro Arg Ser Gln Ala Val Ala Arg Ala Arg Val Ser Leu Leu
            180                 185                 190

Arg Ser Ser Leu Arg Phe Ser Ile Ser Tyr Arg Arg Leu Asp Arg Pro
        195                 200                 205

Thr Arg Ile Arg Phe Ser Asp Ser Asn Gly Ser Val Leu Phe Glu His
    210                 215                 220

Pro Ala Ala Pro Thr Gln Asp Gly Leu Val Cys Gly Val Trp Arg Ala
225                 230                 235                 240
```

```
Val Pro Arg Leu Ser Leu Arg Leu Leu Arg Ala Glu Gln Leu His Val
                245                 250                 255

Ala Leu Val Thr Leu Thr His Pro Ser Gly Glu Val Trp Gly Pro Leu
            260                 265                 270

Ile Arg His Arg Ala Leu Ala Ala Glu Thr Phe Ser Ala Ile Leu Thr
        275                 280                 285

Leu Glu Gly Pro Pro Gln Gln Gly Val Gly Gly Ile Thr Leu Leu Thr
    290                 295                 300

Leu Ser Asp Thr Glu Asp Ser Leu His Phe Leu Leu Leu Phe Arg Gly
305                 310                 315                 320

Leu Leu Glu Pro Arg Ser Gly Gly Leu Thr Gln Val Pro Leu Arg Leu
                325                 330                 335

Gln Ile Leu His Gln Gly Gln Leu Leu Arg Glu Leu Gln Ala Asn Val
            340                 345                 350

Ser Ala Gln Glu Pro Gly Phe Ala Glu Val Leu Pro Asn Leu Thr Val
        355                 360                 365

Gln Glu Met Asp Trp Leu Val Leu Gly Glu Leu Gln Met Ala Leu Glu
    370                 375                 380

Trp Ala Gly Arg Pro Gly Leu Arg Ile Ser Gly His Ile Ala Ala Arg
385                 390                 395                 400

Lys Ser Cys Asp Val Leu Gln Ser Val Leu Cys Gly Ala Asp Ala Leu
                405                 410                 415

Ile Pro Val Gln Thr Gly Ala Ala Gly Ser Ala Ser Leu Thr Leu Leu
            420                 425                 430

Gly Asn Gly Ser Leu Ile Tyr Gln Val Gln Val Val Gly Thr Ser Ser
        435                 440                 445

Glu Val Val Ala Met Thr Leu Glu Thr Lys Pro Gln Arg Arg Asp Gln
    450                 455                 460
```

Arg Thr Val Leu Cys His Met Ala Gly Leu Gln Pro Gly Gly His Thr
465 470 475 480

Ala Val Gly Ile Cys Pro Gly Leu Gly Ala Arg Gly Ala His Met Leu
485 490 495

Leu Gln Asn Glu Leu Phe Leu Asn Val Gly Thr Lys Asp Phe Pro Asp
500 505 510

Gly Glu Leu Arg Gly His Val Ala Ala Leu Pro Tyr Cys Gly His Ser
515 520 525

Ala Arg His Asp Thr Leu Pro Val Pro Leu Ala Gly Ala Leu Val Leu
530 535 540

Pro Pro Val Lys Ser Gln Ala Ala Gly His Ala Trp Leu Ser Leu Asp
545 550 555 560

Thr His Cys His Leu His Tyr Glu Val Leu Leu Ala Gly Leu Gly Gly
565 570 575

Ser Glu Gln Gly Thr Val Thr Ala His Leu Leu Gly Pro Pro Gly Thr
580 585 590

Pro Gly Pro Arg Arg Leu Leu Lys Gly Phe Tyr Gly Ser Glu Ala Gln
595 600 605

Gly Val Val Lys Asp Leu Glu Pro Glu Leu Leu Arg His Leu Ala Lys
610 615 620

Gly Met Ala Ser Leu Leu Ile Thr Thr Lys Gly Ser Pro Arg Gly Glu
625 630 635 640

Leu Arg Gly Gln Val His Ile Ala Asn Gln Cys Glu Val Gly Gly Leu
645 650 655

Arg Leu Glu Ala Ala Gly Ala Glu Gly Val Arg Ala Leu Gly Ala Pro
660 665 670

Asp Thr Ala Ser Ala Ala Pro Pro Val Val Pro Gly Leu Pro Ala Leu
675 680 685

```
Ala Pro Ala Lys Pro Gly Gly Pro Gly Arg Pro Arg Asp Pro Asn Thr
    690                 695                 700

Cys Phe Phe Glu Gly Gln Gln Arg Pro His Gly Ala Arg Trp Ala Pro
705                 710                 715                 720

Asn Tyr Asp Pro Leu Cys Ser Leu Cys Thr Cys Gln Arg Arg Thr Val
                725                 730                 735

Ile Cys Asp Pro Val Val Cys Pro Pro Pro Ser Cys Pro His Pro Val
            740                 745                 750

Gln Ala Pro Asp Gln Cys Cys Pro Val Cys Pro Glu Lys Gln Asp Val
        755                 760                 765

Arg Asp Leu Pro Gly Leu Pro Arg Ser Arg Asp Pro Gly Glu Gly Cys
    770                 775                 780

Tyr Phe Asp Gly Asp Arg Ser Trp Arg Ala Ala Gly Thr Arg Trp His
785                 790                 795                 800

Pro Val Val Pro Pro Phe Gly Leu Ile Lys Cys Ala Val Cys Thr Cys
                805                 810                 815

Lys Gly Gly Thr Gly Glu Val His Cys Glu Lys Val Gln Cys Pro Arg
            820                 825                 830

Leu Ala Cys Ala Gln Pro Val Arg Val Asn Pro Thr Asp Cys Cys Lys
        835                 840                 845

Gln Cys Pro Val Gly Ser Gly Ala His Pro Gln Leu Gly Asp Pro Met
    850                 855                 860

Gln Ala Asp Gly Pro Arg Gly Cys Arg Phe Ala Gly Gln Trp Phe Pro
865                 870                 875                 880

Glu Ser Gln Ser Trp His Pro Ser Val Pro Pro Phe Gly Glu Met Ser
                885                 890                 895

Cys Ile Thr Cys Arg Cys Gly Ala Gly Val Pro His Cys Glu Arg Asp
```

900 905 910

Asp Cys Ser Leu Pro Leu Ser Cys Gly Ser Gly Lys Glu Ser Arg Cys
915 920 925

Cys Ser Arg Cys Thr Ala His Arg Arg Pro Ala Pro Glu Thr Arg Thr
930 935 940

Asp Pro Glu Leu Glu Lys Glu Ala Glu Gly Ser
945 950 955

<210> 9

<211> 3299

<212> DNA

<213> Homo sapiens

<220>

<221> source

<222> (1)..(3299)

<223> small intestine

<220>

<221> gene

<222> (1)..(3299)

<223> DRM

<220>

<221> CDS

<222> (130)..(684)

<223> DRM

<300>

<301> Topol, L.Z., Modi, W.S., Koochekpour, S., and Blair, D.G.

<302> DRM/Gremlin (CKTSF1B1) maps to human chromosome 15 adn is highly expressed in adult and fetal brain

<303> Cytogenet. Cell Genet.

<304> 89

<305> 1

<306> 79-84

<307> 2000

<308> AF154054

<309> 2000-10-18

<313> (1)..(3299)

<300>

<301> Topol, L.Z., Marx, M., Calothy, G., and Blair, D.G.

<302> Direct Submission

<303> Oncogene Mechanisms Section, Basic Research Laboratory, NIH/NCI

<304> 1

<305> 1

<306> 1

<307> 1999-05-25

<308> AF154054

<309> 2000-10-18

<313> (1)..(3299)

<400> 9
ataataatta ggccaagcgt tgaatagtac gggggggggg gggggcgag ccccggcggc
60

tctggccgcg gccgcactca gcgccacgcg tcgaaagcgc aggccccgag gacccgccgc
120

```
actgacagt atg agc cgc aca gcc tac acg gtg gga gcc ctg ctt ctc ctc
171
          Met Ser Arg Thr Ala Tyr Thr Val Gly Ala Leu Leu Leu Leu
          1               5                   10

ttg ggg acc ctg ctg ccg gct gct gaa ggg aaa aag aaa ggg tcc caa
219
Leu Gly Thr Leu Leu Pro Ala Ala Glu Gly Lys Lys Lys Gly Ser Gln
15                  20                  25                  30

ggt gcc atc ccc ccg cca gac aag gcc cag cac aat gac tca gag cag
267
Gly Ala Ile Pro Pro Pro Asp Lys Ala Gln His Asn Asp Ser Glu Gln
                35                  40                  45

act cag tcg ccc cag cag cct ggc tcc agg aac cgg ggg cgg ggc caa
315
Thr Gln Ser Pro Gln Gln Pro Gly Ser Arg Asn Arg Gly Arg Gly Gln
            50                  55                  60

ggg cgg ggc act gcc atg ccc ggg gag gag gtg ctg gag tcc agc caa
363
Gly Arg Gly Thr Ala Met Pro Gly Glu Glu Val Leu Glu Ser Ser Gln
        65                  70                  75

gag gcc ctg cat gtg acg gag cgc aaa tac ctg aag cga gac tgg tgc
411
Glu Ala Leu His Val Thr Glu Arg Lys Tyr Leu Lys Arg Asp Trp Cys
    80                  85                  90

aaa acc cag ccg ctt aag cag acc atc cac gag gaa ggc tgc aac agt
459
Lys Thr Gln Pro Leu Lys Gln Thr Ile His Glu Glu Gly Cys Asn Ser
95                  100                 105                 110

cgc acc atc atc aac cgc ttc tgt tac ggc cag tgc aac tct ttc tac
507
Arg Thr Ile Ile Asn Arg Phe Cys Tyr Gly Gln Cys Asn Ser Phe Tyr
                115                 120                 125

atc ccc agg cac atc cgg aag gag gaa ggt tcc ttt cag tcc tgc tcc
555
Ile Pro Arg His Ile Arg Lys Glu Glu Gly Ser Phe Gln Ser Cys Ser
            130                 135                 140

ttc tgc aag ccc aag aaa ttc act acc atg atg gtc aca ctc aac tgc
603
Phe Cys Lys Pro Lys Lys Phe Thr Thr Met Met Val Thr Leu Asn Cys
        145                 150                 155

cct gaa cta cag cca cct acc aag aag aag aga gtc aca cgt gtg aag
651
Pro Glu Leu Gln Pro Pro Thr Lys Lys Lys Arg Val Thr Arg Val Lys
    160                 165                 170
```

cag tgt cgt tgc ata tcc atc gat ttg gat taa gccaaatcca ggtgcaccca
704
Gln Cys Arg Cys Ile Ser Ile Asp Leu Asp
175 180

gcatgtccta ggaatgcaga cccaggaagt cccagaccta aaacaaccag attcttactt
764

ggcttaaacc tagaggccag aagaaccccc agctgcctcc tggcaggagc ctgcttgtgc
824

gtagttcgtg tgcatgagtg tggatgggtg cctgtgggtg tttttagaca ccagagaaaa
884

cacagtctct gctagagagc acttcctatt ttgtaaacct atctgcttta atggggatgt
944

accagaaacc cacctcaccc cggctcacat ctaaaggggc ggggccgtgg tctggttctg
1004

actttgtgtt tttgtgccct cctggggacc agaatctcct ttcggaatga atgttcatgg
1064

aagaggctcc tctgagggca agagacctgt tttagtgctg cattcgacat ggaaaagtcc
1124

ttttaacctg tgcttgcatc ctcctttcct cctcctcctc acaatccatc tcttcttaag
1184

ttgacagtga ctatgtcagt ctaatctctt gtttgccagg gttcctaaat taattcactt
1244

aaccatgatg caaatgtttt tcatttggtg aagacctcca gactctggga gaggctggtg
1304

tgggcaagga caagcaggat agtggagtga gaaagggagg gtggagggtg aggccaaatc
1364

aggtccagca aaagtcagta gggacattgc agaagcttga aaggccaata ccagaacaca
1424

ggctgatgct tctgagaaag tcttttccta gtatttaaca aaacccaagt gaacagagga
1484

gaaatgagat tgccagaaag tgattaactt tggccgttgc aatctgctca aacctaacac
1544

caaactgaaa acataaatac tgaccactcc tatgttcgga cccaagcaag ttagctaaac
1604

caaaccaact cctctgcttt gtccctcagg tggaaaagag aggtagttta gaactctctg
1664

catagggggtg ggaattaatc aaaaacctca gaggctgaaa ttcctaatac ctttccttta 1724

tcgtggttat agtcagctca tttccattcc actatttccc ataatgcttc tgagagccac 1784

taacttgatt gataaagatc ctgcctctgc tgagtgtacc tgacagtagt ctaagatgag 1844

agagtttagg gactactctg ttttaacaag aaatattttg gggtcttttt tgttttaact 1904

attgtcagga gattgggcta aagagaagac gacgagagta aggaaataaa gggaattgcc 1964

tctggctaga gagtagttag gtgttaatac ctggtagaga tgtaagggat atgacctccc 2024

tttctttatg tgctcacttg aggatctgag gggaccctgt taggagagca tagcatcatg 2084

atgtattagc tgttcatctg ctactggttg gatggacata actattgtaa ctattcagta 2144

tttactggta ggcactgtcc tctgattaaa cttggcctac tggcaatggc tacttaggat 2204

tgatctaagg gccaaagtgc agggtgggtg aactttattg tactttggat ttggttaacc 2264

tgttttcctc aagcctgagg ttttatatac aaactccctg aatactcttt ttgccttgtt 2324

acttctcagc ctcctagcca agtcctatgt aatatggaaa acaaacactg cagacttgag 2384

attcagttgc cgatcaaggc tctggcattc agagaaccct tgcaactcga gaagctgttt 2444

ttgatttcgt ttttgttttg aaccggtgct ctcccatcta acaactaaca aggaccattt 2504

ccaggcggga gatattttaa acacccaaaa tgttgggtct gatttccaaa cttttaaact 2564

cactactgat gattctcacg ctaggcgaat ttgtccaaac acatagtgtg tgtgttttgt 2624

atacactgta tgaccccacc ccaaatcttt gtattgtcca cattctccaa caataaagca 2684

cagagtggat ttaattaagc acacaaatgc taaggcagaa ttttgagggt gggagagaag 2744

aaaagggaaa gaagctgaaa atgtaaaacc acaccaggga ggaaaaatga cattcagaac
2804

caccaaacac tgaatttctc ttgttgtttt aactctccca caagaatgca atttcgttaa
2864

tggagatgac ttaagttggc agcagtaatc ttcttttagg agcttgtacc acagtcttgc
2924

acataagtgc agatttgccc caagtaaaga gaatttcctc aacactaact tcacggggat
2984

aatcaccacg taactaccct taaagcatat cactagccaa agaggggaat atctgttctt
3044

cttactgtgc ctatattaag actagtacaa atgtggtgtg tcttccaact ttcattgaaa
3104

atgccatatc tataccatat tttattcgag tcactgatga tgtaatgata tatttttca
3164

ttattatagt agaatatttt tatggcaaga gatttgtggt cttgatcata cctattaaaa
3224

taatgccaaa caccaaatat gaattttatg atgtacactt tgtgcttggc attaaaagaa
3284

aaaaacacac acgcc
3299

<210> 10

<211> 184

<212> PRT

<213> Homo sapiens

<400> 10

Met Ser Arg Thr Ala Tyr Thr Val Gly Ala Leu Leu Leu Leu Gly
1 5 10 15

Thr Leu Leu Pro Ala Ala Glu Gly Lys Lys Lys Gly Ser Gln Gly Ala
20 25 30

Ile Pro Pro Pro Asp Lys Ala Gln His Asn Asp Ser Glu Gln Thr Gln
35 40 45

Ser Pro Gln Gln Pro Gly Ser Arg Asn Arg Gly Arg Gly Gln Gly Arg
50 55 60

Gly Thr Ala Met Pro Gly Glu Glu Val Leu Glu Ser Ser Gln Glu Ala
65 70 75 80

Leu His Val Thr Glu Arg Lys Tyr Leu Lys Arg Asp Trp Cys Lys Thr
85 90 95

Gln Pro Leu Lys Gln Thr Ile His Glu Glu Gly Cys Asn Ser Arg Thr
100 105 110

Ile Ile Asn Arg Phe Cys Tyr Gly Gln Cys Asn Ser Phe Tyr Ile Pro
115 120 125

Arg His Ile Arg Lys Glu Glu Gly Ser Phe Gln Ser Cys Ser Phe Cys
130 135 140

Lys Pro Lys Lys Phe Thr Thr Met Met Val Thr Leu Asn Cys Pro Glu
145 150 155 160

Leu Gln Pro Pro Thr Lys Lys Lys Arg Val Thr Arg Val Lys Gln Cys
165 170 175

Arg Cys Ile Ser Ile Asp Leu Asp
180

<210> 11

<211> 804

<212> DNA

<213> Homo sapiens

<220>

<221> source

<222> (1)..(804)

<223>

<220>

<221> gene

<222> (1)..(804)

<223> CER1

<220>

<221> CDS

<222> (1)..(804)

<223> cerberus-related 1; cerberus 1 (Xenopus laevis) homolog (cysteine knot superfamily)

<220>

<221> misc_feature

<222> (361)..(741)

<223> DAN domain

<220>

<221> misc_feature

<222> (484)..(723)

<223> Cysteine knot region

<220>

<221> misc_feature

<222> (490)..(723)

<223> C-terminal cysteine knot-like domain

<300>

<301> Lah, M., Brodnicki, T., Maccarone, P., Nash, A., Stanley, E., and Harvey, R.P.

<302> Human cerberus related gene CER1 maps to chromosome 9

<303> Genomics

<304> 55

<305> 3

<306> 364-366

<307> 1999

<308> NM_005454

<309> 2001-12-20

<313> (1)..(804)

<400> 11

```
atg cat ctc ctc tta ttt cag ctg ctg gta ctc ctg cct cta gga aag
48
Met His Leu Leu Leu Phe Gln Leu Leu Val Leu Leu Pro Leu Gly Lys
1               5                  10                  15

acc aca cgg cac cag gat ggc cgc cag aat cag agt tct ctt tcc ccc
96
Thr Thr Arg His Gln Asp Gly Arg Gln Asn Gln Ser Ser Leu Ser Pro
            20                  25                  30

gta ctc ctg cca agg aat caa aga gag ctt ccc aca ggc aac cat gag
144
Val Leu Leu Pro Arg Asn Gln Arg Glu Leu Pro Thr Gly Asn His Glu
        35                  40                  45

gaa gct gag gag aag cca gat ctg ttt gtc gca gtg cca cac ctt gta
192
Glu Ala Glu Glu Lys Pro Asp Leu Phe Val Ala Val Pro His Leu Val
    50                  55                  60

gcc acc agc cct gca ggg gaa ggc cag agg cag aga gag aag atg ctg
240
Ala Thr Ser Pro Ala Gly Glu Gly Gln Arg Gln Arg Glu Lys Met Leu
65                  70                  75                  80

tcc aga ttt ggc agg ttc tgg aag aag cct gag aga gaa atg cat cca
288
Ser Arg Phe Gly Arg Phe Trp Lys Lys Pro Glu Arg Glu Met His Pro
                85                  90                  95
```

```
tcc agg gac tca gat agt gag ccc ttc cca cct ggg acc cag tcc ctc
336
Ser Arg Asp Ser Asp Ser Glu Pro Phe Pro Pro Gly Thr Gln Ser Leu
            100                 105                 110

atc cag ccg ata gat gga atg aaa atg gag aaa tct cct ctt cgg gaa
384
Ile Gln Pro Ile Asp Gly Met Lys Met Glu Lys Ser Pro Leu Arg Glu
        115                 120                 125

gaa gcc aag aaa ttc tgg cac cac ttc atg ttc aga aaa act ccg gct
432
Glu Ala Lys Lys Phe Trp His His Phe Met Phe Arg Lys Thr Pro Ala
    130                 135                 140

tct cag ggg gtc atc ttg ccc atc aaa agc cat gaa gta cat tgg gag
480
Ser Gln Gly Val Ile Leu Pro Ile Lys Ser His Glu Val His Trp Glu
145                 150                 155                 160

acc tgc agg aca gtg ccc ttc agc cag act ata acc cac gaa ggc tgt
528
Thr Cys Arg Thr Val Pro Phe Ser Gln Thr Ile Thr His Glu Gly Cys
                165                 170                 175

gaa aaa gta gtt gtt cag aac aac ctt tgc ttt ggg aaa tgc ggg tct
576
Glu Lys Val Val Val Gln Asn Asn Leu Cys Phe Gly Lys Cys Gly Ser
            180                 185                 190

gtt cat ttt cct gga gcc gcg cag cac tcc cat acc tcc tgc tct cac
624
Val His Phe Pro Gly Ala Ala Gln His Ser His Thr Ser Cys Ser His
        195                 200                 205

tgt ttg cct gcc aag ttc acc acg atg cac ttg cca ctg aac tgc act
672
Cys Leu Pro Ala Lys Phe Thr Thr Met His Leu Pro Leu Asn Cys Thr
    210                 215                 220

gaa ctt tcc tcc gtg atc aag gtg gtg atg ctg gtg gag gag tgc cag
720
Glu Leu Ser Ser Val Ile Lys Val Val Met Leu Val Glu Glu Cys Gln
225                 230                 235                 240

tgc aag gtg aag acg gag cat gaa gat gga cac atc cta cat gct ggc
768
Cys Lys Val Lys Thr Glu His Glu Asp Gly His Ile Leu His Ala Gly
                245                 250                 255

tcc cag gat tcc ttt atc cca gga gtt tca gct tga
804
Ser Gln Asp Ser Phe Ile Pro Gly Val Ser Ala
            260                 265
```

<210> 12

<211> 267

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<222> (361)..(741)

<223> DAN domain

<220>

<221> misc_feature

<222> (484)..(723)

<223> Cysteine knot region

<220>

<221> misc_feature

<222> (490)..(723)

<223> C-terminal cysteine knot-like domain

<400> 12

Met His Leu Leu Leu Phe Gln Leu Leu Val Leu Leu Pro Leu Gly Lys
1 5 10 15

Thr Thr Arg His Gln Asp Gly Arg Gln Asn Gln Ser Ser Leu Ser Pro
20 25 30

Val Leu Leu Pro Arg Asn Gln Arg Glu Leu Pro Thr Gly Asn His Glu
35 40 45

Glu Ala Glu Glu Lys Pro Asp Leu Phe Val Ala Val Pro His Leu Val
50 55 60

Ala Thr Ser Pro Ala Gly Glu Gly Gln Arg Gln Arg Glu Lys Met Leu

65 70 75 80

Ser Arg Phe Gly Arg Phe Trp Lys Lys Pro Glu Arg Glu Met His Pro
85 90 95

Ser Arg Asp Ser Asp Ser Glu Pro Phe Pro Pro Gly Thr Gln Ser Leu
100 105 110

Ile Gln Pro Ile Asp Gly Met Lys Met Glu Lys Ser Pro Leu Arg Glu
115 120 125

Glu Ala Lys Lys Phe Trp His His Phe Met Phe Arg Lys Thr Pro Ala
130 135 140

Ser Gln Gly Val Ile Leu Pro Ile Lys Ser His Glu Val His Trp Glu
145 150 155 160

Thr Cys Arg Thr Val Pro Phe Ser Gln Thr Ile Thr His Glu Gly Cys
165 170 175

Glu Lys Val Val Val Gln Asn Asn Leu Cys Phe Gly Lys Cys Gly Ser
180 185 190

Val His Phe Pro Gly Ala Ala Gln His Ser His Thr Ser Cys Ser His
195 200 205

Cys Leu Pro Ala Lys Phe Thr Thr Met His Leu Pro Leu Asn Cys Thr
210 215 220

Glu Leu Ser Ser Val Ile Lys Val Val Met Leu Val Glu Glu Cys Gln
225 230 235 240

Cys Lys Val Lys Thr Glu His Glu Asp Gly His Ile Leu His Ala Gly
245 250 255

Ser Gln Asp Ser Phe Ile Pro Gly Val Ser Ala
260 265

<210> 13

<211> 2032

<212> DNA

<213> Homo sapiens

<220>

<221> source

<222> (1)..(2032)

<223> Homo sapiens: Taxon:9606

<220>

<221> gene

<222> (1)..(2032)

<223> BMPR1B

<220>

<221> CDS

<222> (274)..(1782)

<223> Serine/threonine receptor kinase

<220>

<221> misc_feature

<222> (367)..(606)

<223> Activin_recp; Region: Activin types I and II

<220>

<221> misc_feature

<222> (883)..(1746)

<223> pkinase; Region: Eukaryotic protein kinase domain

<220>

<221> misc_feature

<222> (883)..(1746)

<223> TyrKc; Region: Tyrosina kinase, catalytic domain

<220>

<221> misc_feature

<222> (883)..(1725)

<223> TKc; Region: Serine/Threonine protein kinases, catalytic domain

<300>

<301> ten Dijke, P., Yamashita, H., Ichijo, H., Franzen, P., Laiho, M., Miyazono, K., and Heldin, C.H.

<302> Characterization of type I receptors for transforming growth factor-beta and activin

<303> Science

<304> 264

<305> 5155

<306> 101-104

<307> 1994

<308> NM_001203

<309> 2000-10-31

<313> (1)..(2032)

<300>

<301> Ide, H., Katoh, M., Sasaki, H., Yoshida, T., Aoki, K., Nawa, Y., Osada, Y., Sugimura, T., and Terada, M.

<302> Cloning of human bone morphogenetic protein type IB receptor (BMPR-IB) and its expression in prostate cancer in comparison with other BMPRs

<303> Oncogene

<304> 14

<305> 11

<306> 1377-1382

<307> 1997

<308> NM_001203

<309> 2000-10-31

<313> (1)..(2032)

<300>

<301> Ide, H., Saito-Ohara, P., Ohnami, S., Osada, Y., Ikeuchi, T., Yoshida, T., and Terada, M.

<302> Assignment of the BMPR1A and BMPR1B genes to human chromosome 10q22.3 and 4q23-->q24 by in situ hybridization and radiation hybrid mapping

<303> Cytogenet. Cell. Genet.

<304> 81

<305> 3

<306> 285-286

<307> 1998

<308> NM_001203

<309> 2000-10-31

<313> (1)..(2032)

<300>

<301> Astrom, A.K., Jin, D., Imamura, T., Roijer, E., Rosenzweig, B., Miyazono, K., ten Dijke, P., and Stenman, G.

<302> Chromosomal localization of three human genes encoding bone morphogenetic protein receptors

<303> Mamm. Genome

<304> 10

<305> 3

<306> 299-302

<307> 1999

<308> NM_001203

<309> 2000-10-31

<313> (1)..(2032)

<400> 13
cgcggggcgc ggagtcggcg gggcctcgcg ggacgcgggc agtgcggaga ccgcggcgct
60

gaggacgcgg gagccgggag cgcacgcgcg gggtggagtt cagcctactc tttcttagat
120

gtgaaaggaa aggaagatca tttcatgcct tgttgataaa ggttcagact tctgctgatt
180

cataaccatt tggctctgag ctatgacaag agaggaaaca aaaagttaaa cttacaagcc
240

tgccataagt gagaagcaaa cttccttgat aac atg ctt ttg cga agt gca gga
294
Met Leu Leu Arg Ser Ala Gly
1 5

aaa tta aat gtg ggc acc aag aaa gag gat ggt gag agt aca gcc ccc
342
Lys Leu Asn Val Gly Thr Lys Lys Glu Asp Gly Glu Ser Thr Ala Pro
10 15 20

acc ccc cgt cca aag gtc ttg cgt tgt aaa tgc cac cac cat tgt cca
390
Thr Pro Arg Pro Lys Val Leu Arg Cys Lys Cys His His His Cys Pro
25 30 35

gaa gac tca gtc aac aat att tgc agc aca gac gga tat tgt ttc acg
438
Glu Asp Ser Val Asn Asn Ile Cys Ser Thr Asp Gly Tyr Cys Phe Thr
40 45 50 55

atg ata gaa gag gat gac tct ggg ttg cct gtg gtc act tct ggt tgc
486
Met Ile Glu Glu Asp Asp Ser Gly Leu Pro Val Val Thr Ser Gly Cys
60 65 70

```
cta gga cta gaa ggc tca gat ttt cag tgt cgg gac act ccc att cct
534
Leu Gly Leu Glu Gly Ser Asp Phe Gln Cys Arg Asp Thr Pro Ile Pro
            75                  80                  85

cat caa aga aga tca att gaa tgc tgc aca gaa agg aac gaa tgt aat
582
His Gln Arg Arg Ser Ile Glu Cys Cys Thr Glu Arg Asn Glu Cys Asn
        90                  95                  100

aaa gac cta cac cct aca ctg cct cca ttg aaa aac aga gat ttt gtt
630
Lys Asp Leu His Pro Thr Leu Pro Pro Leu Lys Asn Arg Asp Phe Val
    105                 110                 115

gat gga cct ata cac cac agg gct tta ctt ata tct gtg act gtc tgt
678
Asp Gly Pro Ile His His Arg Ala Leu Leu Ile Ser Val Thr Val Cys
120                 125                 130                 135

agt ttg ctc ttg gtc ctt atc ata tta ttt tgt tac ttc cgg tat aaa
726
Ser Leu Leu Leu Val Leu Ile Ile Leu Phe Cys Tyr Phe Arg Tyr Lys
                140                 145                 150

aga caa gaa acc aga cct cga tac agc att ggg tta gaa cag gat gaa
774
Arg Gln Glu Thr Arg Pro Arg Tyr Ser Ile Gly Leu Glu Gln Asp Glu
            155                 160                 165

act tac att cct cct gga gaa tcc ctg aga gac tta att gag cag tct
822
Thr Tyr Ile Pro Pro Gly Glu Ser Leu Arg Asp Leu Ile Glu Gln Ser
        170                 175                 180

cag agc tca gga agt gga tca ggc ctc cct ctg ctg gtc caa agg act
870
Gln Ser Ser Gly Ser Gly Ser Gly Leu Pro Leu Leu Val Gln Arg Thr
    185                 190                 195

ata gct aag cag att cag atg gtg aaa cag att gga aaa ggt cgc tat
918
Ile Ala Lys Gln Ile Gln Met Val Lys Gln Ile Gly Lys Gly Arg Tyr
200                 205                 210                 215

ggg gaa gtt tgg atg gga aag tgg cgt ggc gaa aag gta gct gtg aaa
966
Gly Glu Val Trp Met Gly Lys Trp Arg Gly Glu Lys Val Ala Val Lys
                220                 225                 230

gtg ttc ttc acc aca gag gaa gcc agc tgg ttc aga gag aca gaa ata
1014
Val Phe Phe Thr Thr Glu Glu Ala Ser Trp Phe Arg Glu Thr Glu Ile
            235                 240                 245
```

```
tat cag aca gtg ttg atg agg cat gaa aac att ttg ggt ttc att gct
1062
Tyr Gln Thr Val Leu Met Arg His Glu Asn Ile Leu Gly Phe Ile Ala
        250                 255                 260

gca gat atc aaa ggg aca ggg tcc tgg acc cag ttg tac cta atc aca
1110
Ala Asp Ile Lys Gly Thr Gly Ser Trp Thr Gln Leu Tyr Leu Ile Thr
    265                 270                 275

gac tat cat gaa aat ggt tcc ctt tat gat tat ctg aag tcc acc acc
1158
Asp Tyr His Glu Asn Gly Ser Leu Tyr Asp Tyr Leu Lys Ser Thr Thr
280                 285                 290                 295

cta gac gct aaa tca atg ctg aag tta gcc tac tct tct gtc agt ggc
1206
Leu Asp Ala Lys Ser Met Leu Lys Leu Ala Tyr Ser Ser Val Ser Gly
                300                 305                 310

tta tgt cat tta cac aca gaa atc ttt agt act caa ggc aaa cca gca
1254
Leu Cys His Leu His Thr Glu Ile Phe Ser Thr Gln Gly Lys Pro Ala
            315                 320                 325

att gcc cat cga gat ctg aaa agt aaa aac att ctg gtg aag aaa aat
1302
Ile Ala His Arg Asp Leu Lys Ser Lys Asn Ile Leu Val Lys Lys Asn
        330                 335                 340

gga act tgc tgt att gct gac ctg ggc ctg gct gtt aaa ttt att agt
1350
Gly Thr Cys Cys Ile Ala Asp Leu Gly Leu Ala Val Lys Phe Ile Ser
    345                 350                 355

gat aca aat gaa gtt gac ata cca cct aac act cga gtt ggc acc aaa
1398
Asp Thr Asn Glu Val Asp Ile Pro Pro Asn Thr Arg Val Gly Thr Lys
360                 365                 370                 375

cgc tat atg cct cca gaa gtg ttg gac gag agc ttg aac aga aat cac
1446
Arg Tyr Met Pro Pro Glu Val Leu Asp Glu Ser Leu Asn Arg Asn His
                380                 385                 390

ttc cag tct tac atc atg gct gac atg tat agt ttt ggc ctc atc ctt
1494
Phe Gln Ser Tyr Ile Met Ala Asp Met Tyr Ser Phe Gly Leu Ile Leu
            395                 400                 405

tgg gag gtt gct agg aga tgt gta tca gga ggt ata gtg gaa gaa tac
1542
Trp Glu Val Ala Arg Arg Cys Val Ser Gly Gly Ile Val Glu Glu Tyr
        410                 415                 420
```

cag ctt cct tat cat gac cta gtg ccc agt gac ccc tct tat gag gac
1590
Gln Leu Pro Tyr His Asp Leu Val Pro Ser Asp Pro Ser Tyr Glu Asp
425 430 435

atg agg gag att gtg tgc atc aag aag tta cgc ccc tca ttc cca aac
1638
Met Arg Glu Ile Val Cys Ile Lys Lys Leu Arg Pro Ser Phe Pro Asn
440 445 450 455

cgg tgg agc agt gat gag tgt cta agg cag atg gga aaa ctc atg aca
1686
Arg Trp Ser Ser Asp Glu Cys Leu Arg Gln Met Gly Lys Leu Met Thr
460 465 470

gaa tgc tgg gct cac aat cct gca tca agg ctg aca gcc ctg cgg gtt
1734
Glu Cys Trp Ala His Asn Pro Ala Ser Arg Leu Thr Ala Leu Arg Val
475 480 485

aag aaa aca ctt gcc aaa atg tca gag tcc cag gac att aaa ctc tga
1782
Lys Lys Thr Leu Ala Lys Met Ser Glu Ser Gln Asp Ile Lys Leu
490 495 500

taggagagga aaagtaagca tctctgcaga aagccaacag gtactcttct gtttgtgggc
1842

agagcaaaag acatcaaata agcatccaca gtacaagcct tgaacatcgt cctgcttccc
1902

agtgggttca gacctcacct ttcagggagc gacctgggca aagacagaga agctcccaga
1962

aggagagatt gatccgtgtc tgtttgtagg cggagaaacc gttgggtaac ttgttcaaga
2022

tatgatgcat
2032

<210> 14

<211> 502

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<222> (367)..(606)

<223> Activin_recp; Region: Activin types I and II

<220>

<221> misc_feature

<222> (883)..(1746)

<223> pkinase; Region: Eukaryotic protein kinase domain

<220>

<221> misc_feature

<222> (883)..(1746)

<223> TyrKc; Region: Tyrosina kinase, catalytic domain

<220>

<221> misc_feature

<222> (883)..(1725)

<223> TKc; Region: Serine/Threonine protein kinases, catalytic domain

<400> 14

```
Met Leu Leu Arg Ser Ala Gly Lys Leu Asn Val Gly Thr Lys Lys Glu
1               5                  10                  15

Asp Gly Glu Ser Thr Ala Pro Thr Pro Arg Pro Lys Val Leu Arg Cys
            20                  25                  30

Lys Cys His His His Cys Pro Glu Asp Ser Val Asn Asn Ile Cys Ser
        35                  40                  45

Thr Asp Gly Tyr Cys Phe Thr Met Ile Glu Glu Asp Asp Ser Gly Leu
    50                  55                  60

Pro Val Val Thr Ser Gly Cys Leu Gly Leu Glu Gly Ser Asp Phe Gln
65                  70                  75                  80

Cys Arg Asp Thr Pro Ile Pro His Gln Arg Arg Ser Ile Glu Cys Cys
                85                  90                  95

Thr Glu Arg Asn Glu Cys Asn Lys Asp Leu His Pro Thr Leu Pro Pro
```

100 105 110

Leu Lys Asn Arg Asp Phe Val Asp Gly Pro Ile His His Arg Ala Leu
115 120 125

Leu Ile Ser Val Thr Val Cys Ser Leu Leu Leu Val Leu Ile Ile Leu
130 135 140

Phe Cys Tyr Phe Arg Tyr Lys Arg Gln Glu Thr Arg Pro Arg Tyr Ser
145 150 155 160

Ile Gly Leu Glu Gln Asp Glu Thr Tyr Ile Pro Pro Gly Glu Ser Leu
165 170 175

Arg Asp Leu Ile Glu Gln Ser Gln Ser Ser Gly Ser Gly Ser Gly Leu
180 185 190

Pro Leu Leu Val Gln Arg Thr Ile Ala Lys Gln Ile Gln Met Val Lys
195 200 205

Gln Ile Gly Lys Gly Arg Tyr Gly Glu Val Trp Met Gly Lys Trp Arg
210 215 220

Gly Glu Lys Val Ala Val Lys Val Phe Phe Thr Thr Glu Glu Ala Ser
225 230 235 240

Trp Phe Arg Glu Thr Glu Ile Tyr Gln Thr Val Leu Met Arg His Glu
245 250 255

Asn Ile Leu Gly Phe Ile Ala Ala Asp Ile Lys Gly Thr Gly Ser Trp
260 265 270

Thr Gln Leu Tyr Leu Ile Thr Asp Tyr His Glu Asn Gly Ser Leu Tyr
275 280 285

Asp Tyr Leu Lys Ser Thr Thr Leu Asp Ala Lys Ser Met Leu Lys Leu
290 295 300

Ala Tyr Ser Ser Val Ser Gly Leu Cys His Leu His Thr Glu Ile Phe
305 310 315 320

Ser Thr Gln Gly Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser Lys
325 330 335

Asn Ile Leu Val Lys Lys Asn Gly Thr Cys Cys Ile Ala Asp Leu Gly
340 345 350

Leu Ala Val Lys Phe Ile Ser Asp Thr Asn Glu Val Asp Ile Pro Pro
355 360 365

Asn Thr Arg Val Gly Thr Lys Arg Tyr Met Pro Pro Glu Val Leu Asp
370 375 380

Glu Ser Leu Asn Arg Asn His Phe Gln Ser Tyr Ile Met Ala Asp Met
385 390 395 400

Tyr Ser Phe Gly Leu Ile Leu Trp Glu Val Ala Arg Arg Cys Val Ser
405 410 415

Gly Gly Ile Val Glu Glu Tyr Gln Leu Pro Tyr His Asp Leu Val Pro
420 425 430

Ser Asp Pro Ser Tyr Glu Asp Met Arg Glu Ile Val Cys Ile Lys Lys
435 440 445

Leu Arg Pro Ser Phe Pro Asn Arg Trp Ser Ser Asp Glu Cys Leu Arg
450 455 460

Gln Met Gly Lys Leu Met Thr Glu Cys Trp Ala His Asn Pro Ala Ser
465 470 475 480

Arg Leu Thr Ala Leu Arg Val Lys Lys Thr Leu Ala Lys Met Ser Glu
485 490 495

Ser Gln Asp Ile Lys Leu
500

<210> 15

<211> 20

<212> DNA

<213> Homo sapiens

<400> 15
acgagagctc tcactggtcc
20

<210> 16

<211> 20

<212> DNA

<213> Homo sapiens

<400> 16
cattccggat tacatgaggg
20